THE INVESTIGATION OF THE PHARMACOLOGICAL ACTIONS OF CERTAIN NEW QUINOLINE AND PYRAZOLINE DERIVATIVES.

by

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PART I.

THE MEASUREMENT OF ACTIVITY OF LOCAL ANAESTHETICS.

PART II.

CHEMICAL CONSTITUTION AND PHARMACOLOGICAL ACTION

PART III.

THE MEASUREMENT OF ACTIVITY OF LOCAL ANAESTHETICS.*

I. INTRODUCTION.

Local anaesthetic properties are possessed by a very large number of compounds, and methods of accurate comparison of their relative potencies are needed, firstly in order to find compounds of clinical importance, and secondly to discover relationships between chemical constitution and pharmacological action. In the latter case it is necessary to use methods which will express the relative potencies of drugs which differ widely in the intensity of their actions.

The following general principles were accepted by the author as guides in the choice of methods.

The three chief preparations on which local anaesthetic activity can be measured are:-

(a) Frog's nerves.
(b) Rabbit's cornea.
(c) Human wheal (intradermal injection).

These/

*For convenience technical details of methods are put in appendix.

These three methods measure different properties.

Paralysis of the frog's sciatic measures two properties of a drug, firstly its power to penetrate nerve sheaths, and secondly its power to paralyse nerves. The anaesthesia of the rabbit's cornea also measures two properties of a drug, firstly its power to penetrate mucous membranes, and secondly its power to paralyse nerve endings. The intradermal method gives a direct measure of the power of a drug to paralyse nerve endings unaffected by its power of penetration.

It is well known that comparisons based on one method may differ widely from those based on another method. For example procaine has an action equal to cocaine when tested by the intradermal method, or by the frog's sciatic method, but is much less powerful when tested by the corneal method. The quinine derivative optochine on the other hand has a powerful local anaesthetic action on the cornea and on the nerve terminations but has no actions on nerve trunks. It is therefore necessary to use two or three different tests, in order to obtain any estimate of the probable clinical value of a new compound.

General/

General experience in methods of biological standardisation has shown that the best method of obtaining a quantitative estimate of the potency of a preparation is to determine what concentration of the drug which is being tested will produce an action exactly equal to that produced by a known concentration of some other drug which is chosen as the standard.

Moreover in all methods of biological standardisation it is necessary to eliminate errors due to individual variations. Individual variation in response to local anaesthetics is as pronounced as in the response to other drugs. Fig. 2 shows the individual variations to frog's sciatic nerves as measured by the author and these results show a standard deviation of about 50% of the median.

Since it is impossible to obtain a uniform biological population, comparisons must be made in one of two ways: (a) by successive tests with different drugs on the same individual, (b) by using sufficient numbers of test animals to obtain averages that are statistically significant.

The simplest method of comparing local anaesthetic potencies is to determine the minimum effective/

effective concentration of drugs. This measurement is however very difficult in practice beaause local anaesthesia is a graded effect, sensation is first dulled and then abolished and it is difficult to determine exactly when full anaesthesia is just produced. In practice it is usual to introduce a time factor and to measure the concentration of drug which produces anaesthesia within a certain time, or which anaesthetises for a certain period.

This raises the question of the relation between concentration and rate of action or duration of action. The determination of relative activities of two different compounds is a very laborious process if it is necessary to find concentrations which produce an exactly equal action and this difficulty is greatly increased if it is necessary to eliminate biological variations by making the number of observations sufficient to provide an average that is reasonably exact when judged by statistical methods.

It is far quicker to measure the rate or duration of action produced by one concentration of each/

each drug and then to calculate the ratios between the times. This method will only provide accurate results however if the relation between concentration (C) and time (T) is a simple one such as is expressed in the case of rate of action by the formula $CT = K$, or in the case of duration of action $KC = T$. If the relation is an exponential one such as $C^n T = K$, then it is clearly unjustifiable to argue that because drug A at concentration C, produces an action in half the time that is produced by drug B at the same concentration, therefore A is twice as strong as B, for if the relation between time and rate of action is $C^{0.5} T=K$, then the concentration of drug B which will be required to produce an action in the same time as that produced by drug A at concentrations C, will be not two but four.

The writer has therefore examined the problem of the relation between concentration and rate, or duration of action in the various methods studied.

Other factors which it is essential to measure in order to discover whether the drug is likely to be of practical clinical value are its power of producing/

producing irritation and its toxicity. The former can be measured both on the rabbit's cornea and on the intradermal wheal and the latter is most conveniently measured on mice.

II. /

II. PARALYSIS OF FROG'S NERVES.

(a) Method.

The sciatic-gastrocnemius preparation from R. esc. Hung. was used. The sheath of the nerve was stripped, the method of application of the drug is shown in Fig. 1. The drug dissolved in saline solution was applied to a short length of the nerve trunk. The muscle was kept in saline solution and the whole arrangement was covered with a moist chamber.

The response of the muscle was measured on a smoked drum by an isometric lever. Motor paralysis of nerves is a progressive process and since there is a wide individual variation in sensitivity, it is somewhat difficult to choose a suitable end-point; the method used was as follows.

The threshold stimulus of the fresh preparation was first determined, this was taken as the stimulus (only break shocks were used) which produced a response between 5 to 10% of the maximum/

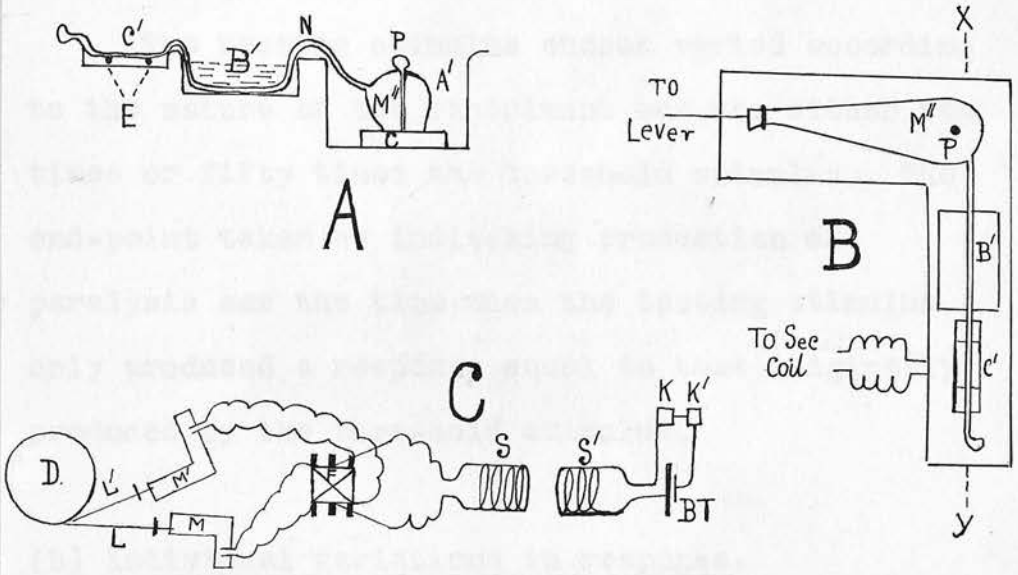


Fig. 1. A. Vertical view of muscle nerve preparation at XY of diagram B.
N = nerve. M'' = muscle. P = pin.
C = cork to fix muscle. E = electrodes from sec.coil
A' = trough for muscle. B' = for drugs.
C' = trough for electrodes.

B. Same as A viewed from top.

C. Scheme for stimulation.
D = redording drum. LL' = levers.
MM' = troughs for both sciatics.
F = commutators. S = sec. coil.
BT = battery. S' = primary coil.
K,K' = keys for break shocks only.

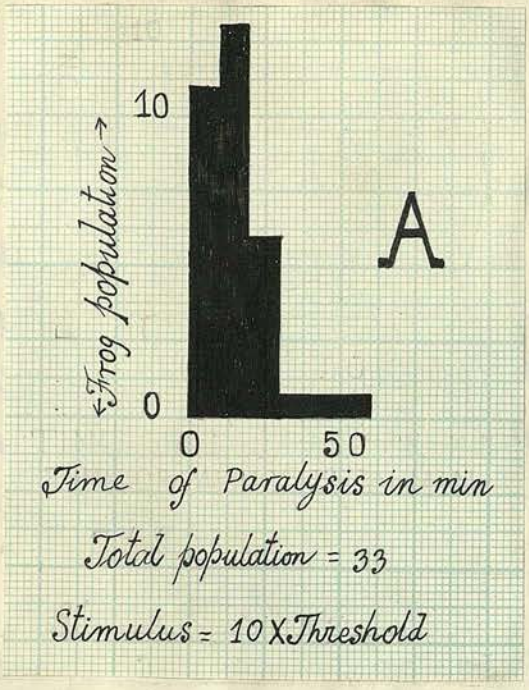
maximum response of the muscle.

The testing stimulus chosen varied according to the nature of the experiment but was either ten times or fifty times the threshold stimulus. The end-point taken as indicating production of paralysis was the time when the testing stimulus only produced a response equal to that originally produced by the threshold stimulus.

(b) Individual variations in response.

The author tested the action of 1% cocaine on 33 preparations. Fig. 2 shows the scatter of the measurements of time onset of paralysis. Statistical analysis of these figures gave the following results.

	Time until paralysis of response to:	
	10 times threshold stimulus (33 experiments)	50 times threshold stimulus (28 experiments)
Mean time in minutes	16	32.1
Standard deviation(σ)	8.75	16.7
Standard deviation of mean σ_m	1.54	3.16


10
0
← Frog population →
A
0
50
Time of Paralysis in min
Total population = 33
Stimulus = 10 X Threshold

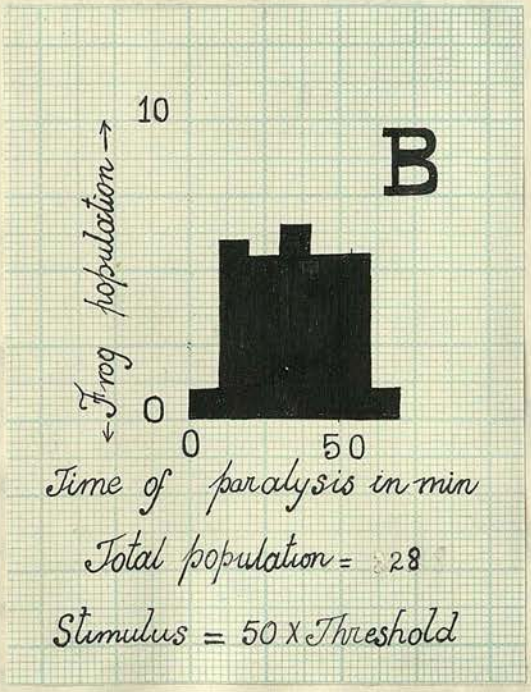

10
0
← Frog population →
B
0
50
Time of paralysis in min
Total population = 28
Stimulus = 50 X Threshold

Fig. 2.

These results show a very wide individual variation for the standard deviations are 55 and 52% of the mean respectively.

Laubender and Ost (1932) have published protocols of figures for the motor paralysis of frog's sciatic nerves by percaine and pantocaine; analysis of these figures gave the following results.

Conc. per cent.	No.of expts.	Time in minutes until paralysis			
		Percaine		Pantocaine	
		Average	Standard deviation σ	Average	Standard deviation σ
0.3	7	65	5	47	13
0.5	9	40	8	40	13
0.75	8	26	4	34	6

These results are more satisfactory than those obtained by the author for the average of the standard deviation is 20% of the mean. In order to obtain satisfactory comparisons it is necessary to obtain averages in which the standard deviation of the mean is not more than 8% for this degree of accuracy only implies that the true result lies almost/

almost certainly within the limits of ± 25% of the mean. If the standard deviation is 20% of the mean about 7 experiments are needed to obtain such an average, whereas if the standard deviation is 50% of the mean, nearly 40 experiments are needed.

These figures are of importance because Rider (1930) concluded that 4 experiments were sufficient to give an accurate measure of the time until paralysis at any concentration. If, however, the standard deviation is as low as 20% of the mean, the mean of 4 experiments will only express a 20 to 1 probability of the true mean lying between 23% of the mean obtained.

The error due to variations cannot be avoided by comparing two drugs one on each of a pair of nerve muscles from the same frog. This fact is shown by the tables given by Laubender and Ost (1932). For example they tested percaine at 0.5% concentration on 9 pairs of nerves; the average time until paralysis was 40 min. in both cases. The ratios obtained between the time of action percaine/pantocaine varied from individual experiments from 0.65 to 1.6. The standard deviation of the mean was 0.33 which is equivalent to 33% and this/

this is a more unfavourable result than was obtained with a single drug acting on nerves from different frogs.

The author obtained very similar results in a comparison of cocaine with a new synthetic drug which was carried out in the same manner. These results are shown in Table I. It will be seen that the standard deviation of the average ratio is 31%, hence 12 experiments would be needed to obtain an average ratio with mean deviation of only 10%.

These results show that accurate values for the rate of paralysis of the frog's sciatic nerves can only be obtained if averages are made from a large number of experiments.

Table I /

Table I.

Time in minutes of onset of anaesthesia in frog's motor nerve.(Cocaine = 1%)

Drug conc. %	Drugs		Time ratio.
	Cocaine	XVI/8	$\frac{\text{Cocaine}}{\text{XVI/8}}$
1	35	15	2.3
	21	10	2.1
	35	28	1.2
	66	34	2.0
	7	5	1.4
	37	22	1.7
	80	25	3.2
			Mean:2.0, σ = 0.62
			σ_η = 0.25
0.5	65	120	$\frac{\text{XVI/8}}{\text{cocaine}}$ = 1.9
	10	44	4.4
	48	54	1.1
	32	60	1.9

Therefore XVI/8 $>1<$ 2 times of cocaine.

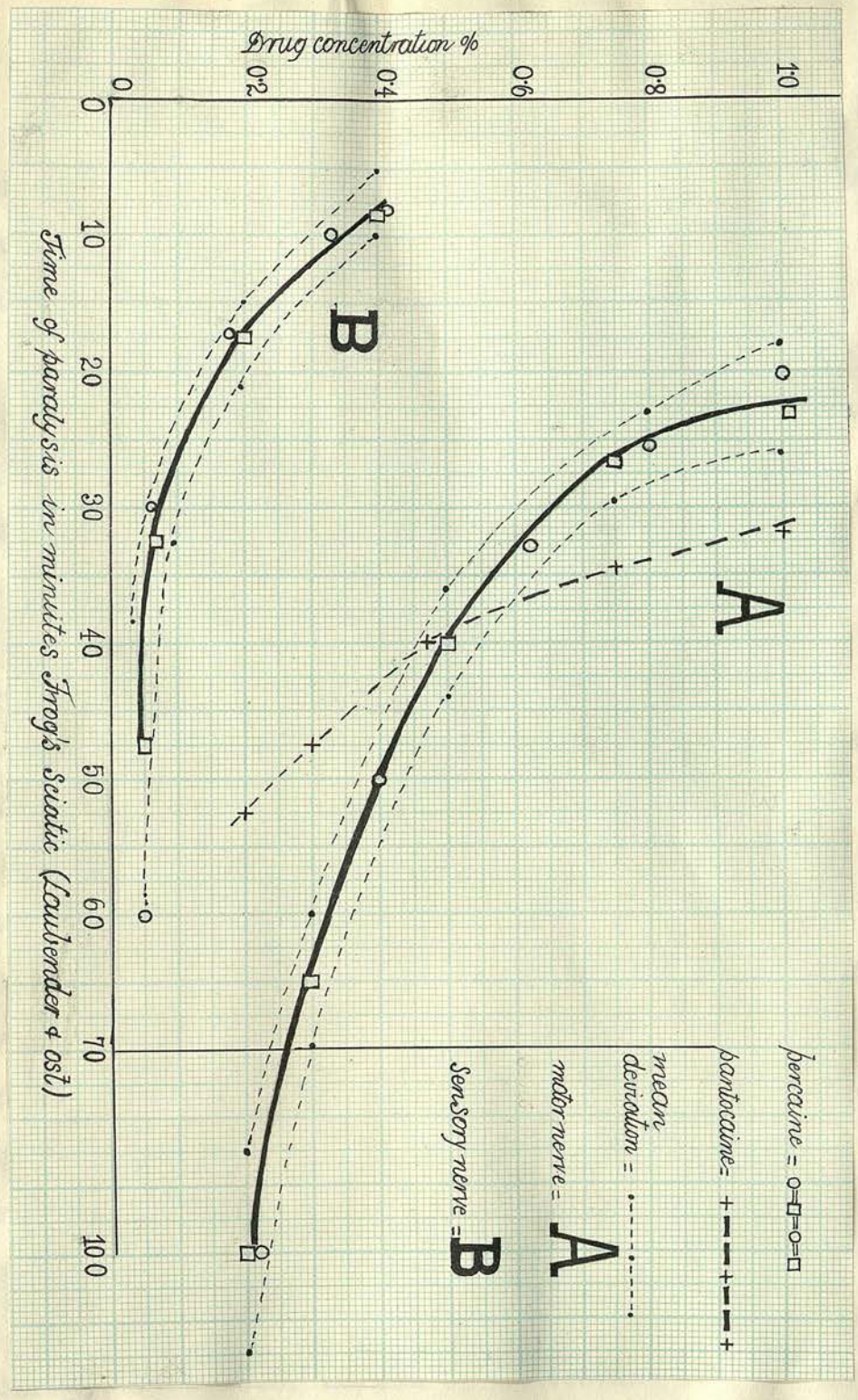
III. RELATION BETWEEN CONCENTRATION AND RATE OF ACTION.

(a) Rate of action on frog's nerves.

Clark (1933a) collected data for the relation between concentration (C) and time (T) until a given action was produced. He showed that the results could be fitted by the general formula C^nT = Constant, but that the value of n varied from 0.3 to 8 and indeed varied widely when a single drug was tested on different types of tissues or organisms.

The author examined the data given by Laubender and Ost (1932) in order to determine whether the rate of paralysis of frog's nerves yielded results of sufficient accuracy to indicate a definite relation between concentration and time until action. Fig. 3 shows these results in the case of percaine. The solid line shows average results whilst the dotted lines are drawn through points measured ± twice mean deviation (σ_m) from the average. Curve A shows the paralysis with motor nerves and curve B the paralysis with sensory nerves.

Fig. 3 /


Drug concentration %
1·0
0·8
0·6
0·4
0·2
0
0
10
20
30
40
50
60
70
80
90
100
Time of paralysis in minutes Frog's Sciatic (Loubender & ost.)
A
B
B
A
B
Sensory nerve
motor nerve
mean deviation
bupivacaine
bercaine = o=□=o=□

The circles show points calculated from the formula C^nT = Constant, where n = 1 and the constants are 20 in the case of Curve A and 3 in the case of Curve B.

The action of pantocaine on the motor nerves follows a completely different course. In this case the simplest formula that gives an approximate fit is $(T-27)C^n = 6$.

These results suffice to show that in the case of the frog's sciatic nerve it is impossible to assume that the ratio between the times at which an action is produced indicates the ratio between the strength of the two drugs. Unfortunately they show in addition that the time at which paralysis is produced is determined by two independent varieties, namely rate of penetration and power to produce action after penetration; hence in the case of percaine and pantocaine (curves A and C) the ratio between the intensity of action varies with every concentration of the drug.

Fig. 3 shows that the curves for the paralysis of motor nerves by pantocaine and percaine respectively cross at a concentration above 0.5%.

At/

At this concentration the activity of the two drugs is equal. With higher concentrations percaine acts the more rapidly, whilst with lower concentrations, pantocaine acts the more rapidly. It is therefore impossible to state whether one drug is more active than the other.

The paralysis of the frog's sciatic nerve cannot therefore serve as a basis for accurate comparisons of the intensity of action of local anaesthetics. It is however a convenient preparation for testing whether a drug does or does not possess any local anaesthetic properties.

(b) Rabbit's cornea.

(i) Method.

The eyes of a rabbit is clipped of its eyelashes and a pocket is made of the lower eyelid, and the cornea flooded with the anaesthetic fluid and kept in contact with the cornea first for five minutes. The sensitivity of the cornea was then measured. If the regult was negative the eye was flooded/

flooded for a second and if necessary for a third period of five minutes. A negative result therefore indicated that a solution did not produce partial anaesthesia after being in contact with the cornea for 15 minutes. The anaesthesia was measured by touching the lower quadrant of the cornea with the head of a pin fairly firmly to note the absence of winking reflex.

Immediately after the onset of winking reflex the eye was thoroughly washed with saline, and the eye was tested from time to time for the return of the reflex to find out the total duration of anaesthesia. The experiments were usually repeated three times and more in case of doubtful anaesthesia (often in lower concentration) to obtain an average result. The same rabbits were used for comparative purposes and the same eye.

(ii) Individual variations in rabbit's cornea.

The author tested the actions of 1% cocaine exposed for 15 minutes on the corneas of a few control animals and obtained the following results.

Rabbit/

concentration, and then a minimum time (T_m) is reached and a further increase of concentration does not reduce the time. The curves can be expressed by the formula $(C - C_m)\ (T - T_m) = \text{Constant}$. In the case of cocaine $C_m = 0.15\%$, $T_m = 0.75$ min. The author however is unable to give the physio-chemical significance of the formula. Fig. 4 shows however that the curves expressing concentration and rate of action for cocaine and drug XVI/15 cross just as did the similar curves for percaine and pantocaine (Fig. 3).

It is therefore impossible to calculate from the curves any definite ratio of activity between the two drugs.

Fig. 4 /

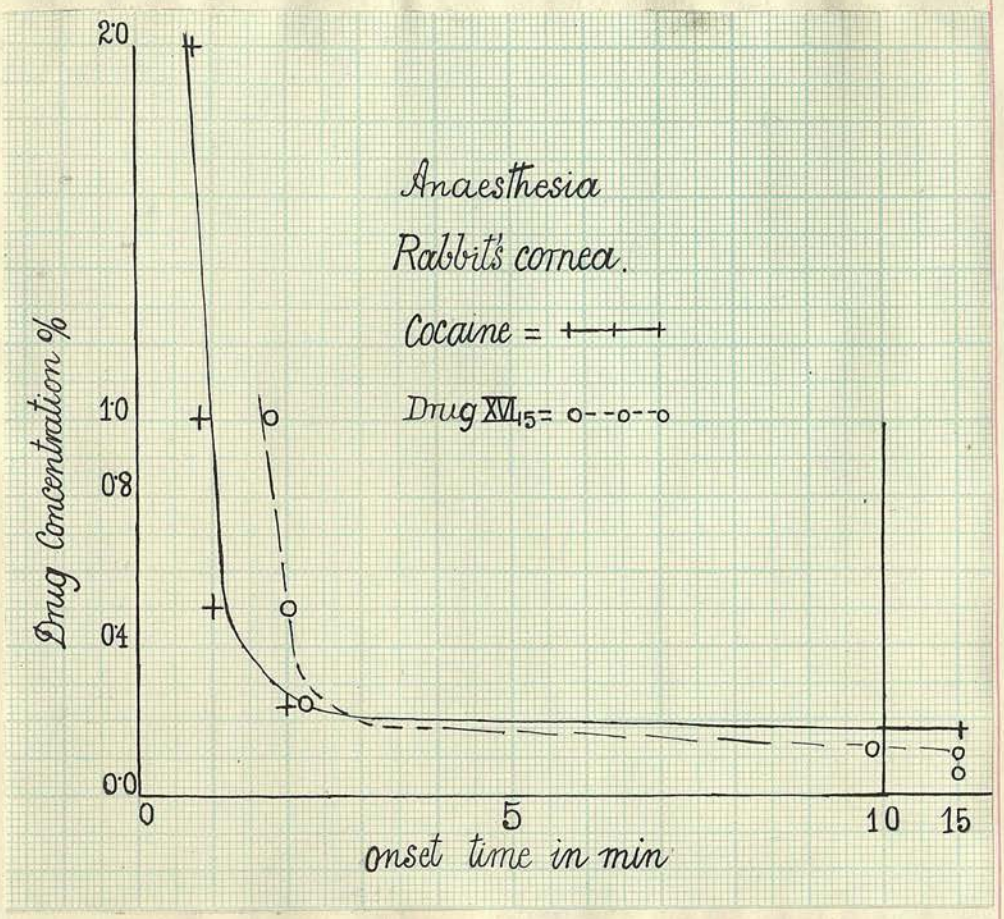

Anaesthesia
Rabbit's cornea.
Cocaine = +—+—+
Drug XVI45 = o--o--o
Drug Concentration %
2·0
1·0
0·8
0·4
0·0
0
5
10
15
onset time in min

Fig. 4.

(c) Human wheal.

Method.

0.25 c.c. of the sterilised isotonic solution to be tested was injected intracutaneously on the flexor surface of the forearm with a 1 c.c. syringe graduated to 1/100 c.c. The duration of anaesthesia was tested by marking the anaesthetic area, and noting the loss of sensation to the more definite sensation of pinprick as compared to the surrounding area of the skin. The end-point was taken when the sensitivity approached to about the sensitivity of the normal surrounding skin. Control injections with only isotonic saline were also tested.

IV. RELATION BETWEEN DRUG CONCENTRATION AND DURATION OF ACTION.

Local anaesthesia both in the rabbit's cornea and in the intradermal wheal is a reversible effect and hence in both cases measurements of the relation between concentration of local anaesthetics and duration of action can be made. In both cases the duration indicates the time taken for the drug to be removed by the circulation. The duration of action of different drugs varies widely and hence the time measured probably expresses the rate of breakdown of some chemical combination rather than a simple process of diffusion.

The author measured the relation between concentration and duration of action of various drugs both on the rabbit's cornea and by the intradermal wheal method.

Tables II and III show the relation obtained between concentration of four drugs and their duration of action on the rabbit's cornea and the human wheal. These results are shown graphically in Figs. 5 and 6. The curves obtained with both preparations/

preparations and all the drugs are similar in type, and resemble hyperbolae.

Various other authors have measured the relation between concentration and duration of action of cocaine on the rabbit's cornea (Bovet 1933, Regnier 1934, Uhlmann 1930) and the human wheal (Sollmann 1914) and for novocaine on the human wheal (Gessner and Klunke 1932, Sollmann 1914). The results obtained agree with those shown by the author if plotted graphically.

The first question is whether there is a simple linear relation between concentration and duration of action. Figs. 5 and 6 show that no such relation exists.

There is however an approximately linear relation between the logarithm of the concentration (C) and the duration (T) . The curves can be expressed by the formula $KC^n = T$ or $\text{Log } K + n\text{Log } C = \text{Log } T$. The slopes of the different drugs differ by n which lies in all cases between 0.5 and 1.0.

Table II /

Table II.

Duration time in minutes. Anaesthesia rabbit's cornea after 15 minutes of exposure to drugs.

Concentration per cent.	Drugs			
	Cocaine	XVI/8	XVII	XVIII/1
1.0	12-15			
0.5	13-15			
0.25	7-8			15-20
0.10		25-30	15-20	10-12
0.05		20-25	10-12	4-6
0.025		10-15	4-6	

Table III /

Table III.

Duration of anaesthesia. Human wheal (time in min.)

Concentration per cent.	Drugs			
	Cocaine	XVI_3	XVII	$XVIII_1$
0.25	15–18	55–75	25–30	20–25*
0.10	7–8	23–30	15–20	15–20
0.05	5–6	20–23	10–15	9–12
0.025	3–5	15–17	8–12	7–8
0.010	incomplete	10–12	4–5	4–5
0.005		6–8		
0.0025		3–5 (incomplete)		

Saline 3–5 min.

* 20–25 min. means that anaesthesia began to disappear after 20 min. and completely disappeared after 25 min.

Table IV.

Relation of concentration and duration of action of local anaesthetics. Formula $t = C^n$ constant $= k$ or $\log t = n. \log C + \log k.$

Drug	Author	n	k
Rabbit's cornea			
Cocaine	Sinha	0.53	18
	Bovet	0.62	20
	Regnier	0.74	45
	Uhlmann	0.40	120
Novocaine	Gessner and Klenke	1.2	2.8
	Uhlmann	0.2	45.0
Larocaine	Gessner and Klenke	0.9	20.0
XVI_8	Sinha	0.57	130
XVII	"	0.95	87
$XVIII_1$	"	0.77	42.5
665	Bovet	0.36	91.0
Percaine	Uhlmann	0.6	1000.0
Tautocaine	Uhlmann	0.37	59.0

Table V.

Human Wheal.

Drug	Author	n	k
Cocaine	Sinha	0.63	42
	Sollmann	0.44	22
Novocaine	Gessner and Klenke	0.64	22
	Sollmann	0.44	14
Larocaine	Sollmann	0.5	28
Pantocaine	Sollmann	0.55	300
XVI_8	Sinha	0.54	80
XVII	"	0.55	50
$XVIII_1$	"	0.55	45
Alypine and β-eucaine	Sollmann	0.44	18

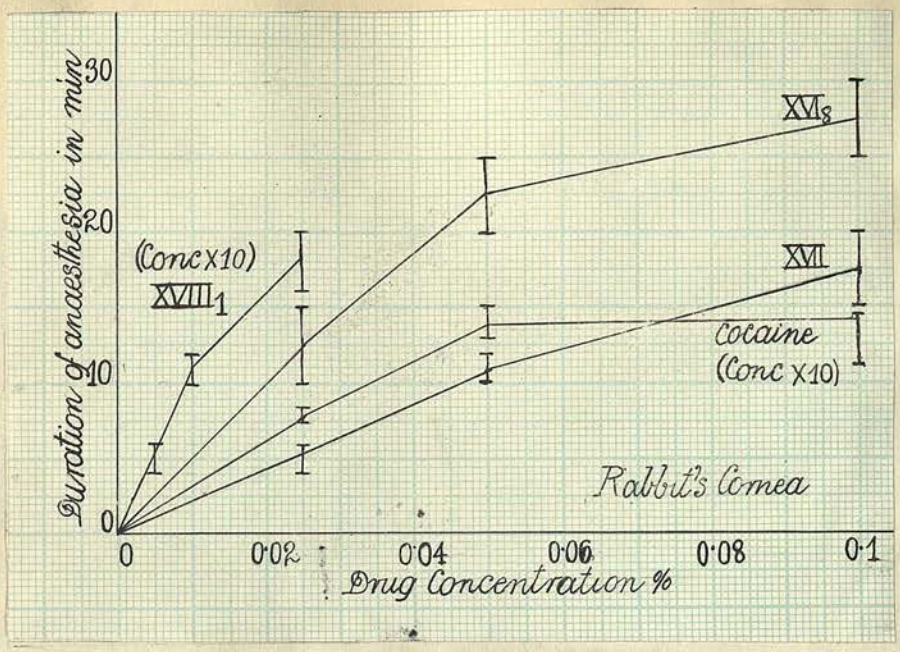

Duration of anaesthesia in min
30
20
10
0
(Conc X10)
XVIII1
XVI8
XVI
cocaine
(Conc X10)
Rabbit's Cornea
0
0·02
0·04
0·06
0·08
0·1
Drug Concentration %

Fig. 5.

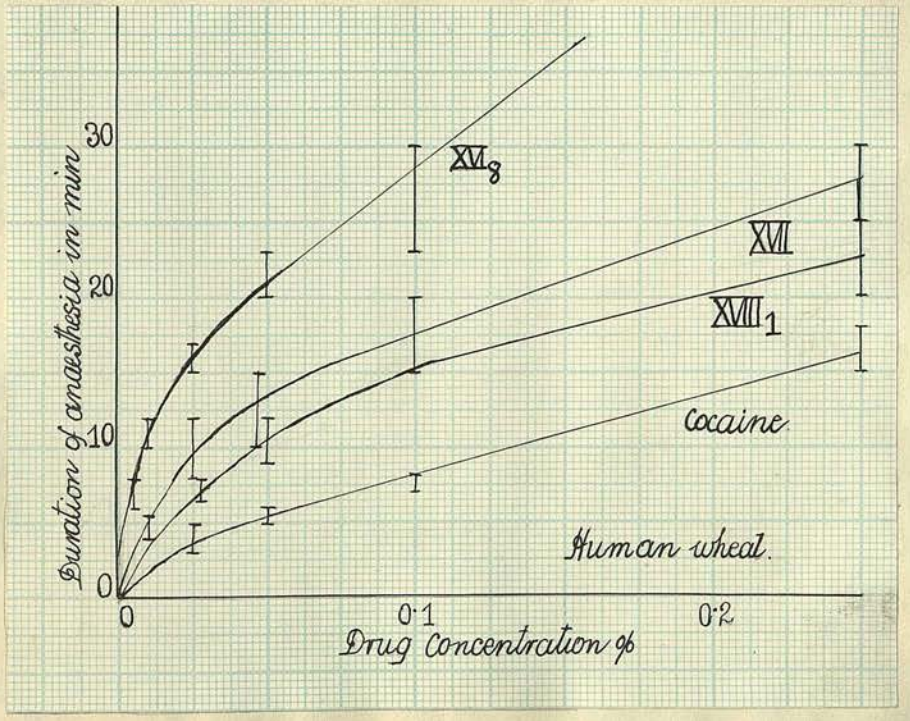
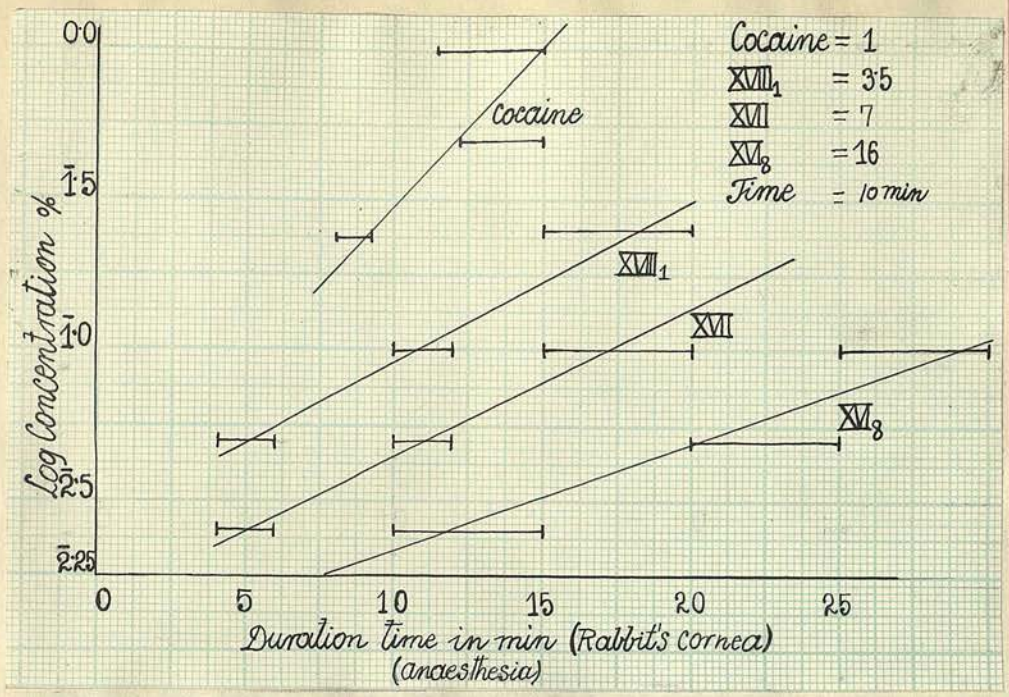
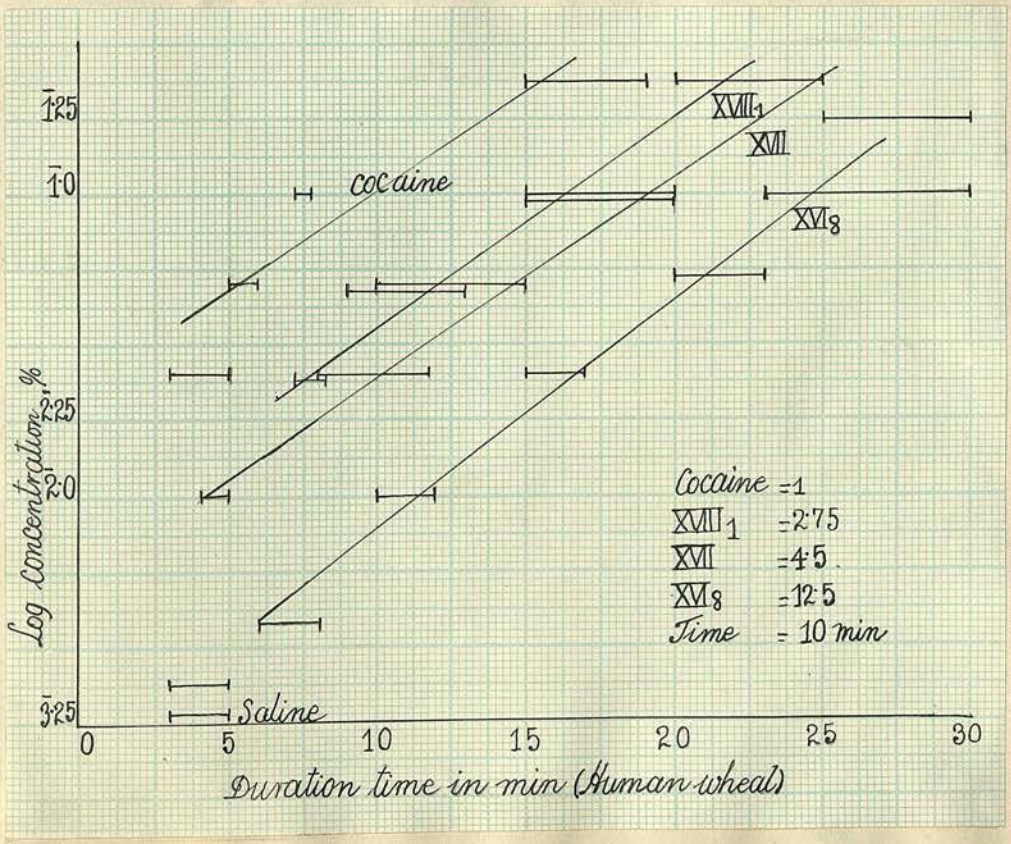

Duration of anaesthesia in min
30
20
10
0
XVI8
XVI
XVIII1
cocaine
Human wheal.
0
0·1
0·2
Drug Concentration %

Fig. 6.


Cocaine = 1
XVIII1 = 3·5
XVII = 7
XVI8 = 16
Time = 10 min
cocaine
XVIII1
XVII
XVI8
Log Concentration %
0·0
1·5
1·0
2·5
2·25
0
5
10
15
20
25
Duration time in min (Rabbit's cornea)
(anaesthesia)


XVIII1
XVII
XVI8
cocaine
Cocaine =1
XVIII1 =2·75
XVII =4·5
XVI8 =12·5
Time = 10 min
Saline
Log Concentration %
1·25
1·0
2·25
2·0
3·25
0
5
10
15
20
25
30
Duration time in min (Human wheal)

Examination of figures (Tables IV and V) obtained by other workers shows a fair agreement in that the duration of action of cocaine both in the rabbit's cornea and on the human wheal can be expressed by the formula $T\sqrt{C} = K = \text{constant}$.

Unfortunately even in the case of cocaine the value of K varies greatly. This is probably due to the different criteria for anaesthesia being employed by different observers. The author is unable to offer any theoretical explanation.

As in the case of the rate of action of drugs so in the case of the duration of action it is only possible to find a satisfactory basis of comparison for the relative potency if the concentration duration curves have a similar slope. This fact is shown by the following example.

Concentrations of drugs producing similar duration of action on the rabbit's cornea.

Drug	Duration 8 min.	Duration 30 min.
(a) cocaine	0.22	2.5
(b) XVII/8	0.04	0.16
Ratio b/a	5.50	15.80

In the case of human wheal the results are more comparable and therefore it would appear that the best method of comparison is to measure the concentration which will produce anaesthesia in human wheal for a standard time, e.g. 10 minutes.

In order to carry out this comparison the duration of anaesthesia in a series of measurements employing different concentrations has been plotted on a logarithmic scale. In this way we obtain a straight line and it is therefore easy to read off the concentration which will give a duration of 10 minutes; these concentrations are then taken as inversely proportional to the efficiency.

Drug Combination and Duration of Action.

Drugs in combination with other drugs are known to affect the duration of action in various ways and both the biological and physical factors are involved. Adrenaline is a drug which is well known to cause enhancement of duration as a result of the vascular constriction which it produces. In clinical application of local anaesthetics adrenaline is/

is frequently used in this way to assist the action.

However the author tested some of the most potent synthetic drugs mentioned above by the wheal method, both with and without adrenaline, and obtained a change in the duration ratio as follows:-

Drug	Concentration	Duration time in min.		Ratio	
		Adrenaline (-)	Adrenaline (+)	(adr-)	(adr+)
Novocaine	1%	18-22	160	1	1
XVI/8	0.1%	20-35	155	1.5	1
XVII	0.25%	22-27	160	1.25	1
$XVIII_1$	0.25%	17-21	75	1.0	0.5

V/

V. DISCUSSION.

Various workers and specially Rider have pointed out that no two drugs can be accurately compared unless both compounds have been tested (a) by the same worker, (b) and by the same technique. The author in this paper has tried to point out the existence of various other factors which make comparison difficult, even when the work is done by the same worker and the same technique. These factors seem to result from the peculiar nature of drug action. Certain of them have here been studied in detail in order that the practical difficulties arising from them might be more readily overcome.

A review of the literature dealing with the accuracy of quantitative tests for local anaesthetics shows that the technique least open to criticism is that of the human wheal, next in order is that involving the rabbit's cornea which gives a somewhat more variable result. It has been generally recognised/

recognised that still more variable results are obtained by the use of frog's sciatic, but this method has been frequently adopted for scientific purposes because the results could be recorded objectively and are not dependent on personal judgment.

Various authors have attempted to reduce the range of variation shown by the results obtained with the frog's sciatic preparation. Rider claims to have reduced the variation to a minimum but even his results show a variation of 90 p.c. in the rate of onset of action of 1 p.c. cocaine.

The results obtained by the author are in harmony with the general experience, variations being greatest in the case of frog's sciatic and much less in the case of cornea and human wheal. For accurate comparisons of two local anaesthetics, the author considers that three or four experiments are sufficient in the case of rabbit's cornea and human wheal, but for frog's sciatic a larger number of experiments should be carried out.

It follows that small variations in the results are not to be regarded as significant; when the differences obtained are significantly great real difference in anaesthetic potency is to be inferred/

inferred. This is specially the case when the results of at least two methods (e.g. cornea and wheal)are available and confirm each other. In discussing the relationship between chemical constitution and pharmacological action, it is important to take into consideration only such differences as have been established beyond any possible doubt, and it is desirable to adhere to the rule of insisting on confirmation by two different methods before using the method as the basis for correlating chemical constitution to pharmacological action.

The data given in this paper, and particularly the concentration-duration curves given in Figs. 5 and 6 show that these curves are hyperbolae. Figs. 7 and 8 show that there is an approximately linear relation between the logarithm of the concentration and the duration of the action. This relation is reminiscent of the well known Weber's law which states that there is a linear relation between the logarithm of the intensity of a stimulus and the physiological effect produced on sense organs.

Clark (1933) has pointed out that in the case of adsorption processes there is a linear relation between the logarithm of the concentration and the

amount/

amount of substance absorbed over a range from 20 to 80 p.c. of the maximum possible adsorption. It is therefore possible that the relation observed between the concentration and duration of action of local anaesthetics is dependent upon the fixation of the drug by the nerve tissue being an adsorption process.

The accuracy of the results is, however, insufficient to make it profitable to attempt to use them as proof of the occurrence of any particular physico-chemical process.

Regarding the significance of the individual variations in frog's sciatic (Fig. 2, A and B), it will be seen that Fig. 2 A. resembles asymmetrical skew type of variation, whereas Fig. 2 B. is more symmetrical and would give a sigmoid type of curve. The author thinks that it is more reasonable to deduce that the change is due to the different intensity of stimulus used (i.e. 10x threshold stimulus for A and 50x threshold stimulus for B.

Clark (1933 e) has explained the cause of such variations as due to (1) the amount of drug the individuals fix and (2) the amount of action they/

action they produce after fixation in cells. If the drug acts quickly as in concentrated solutions the drug will give a skew variation while the same drug in lower concentrations will tend to give a sigmoid type of variation.

The difference here made is not in the concentration of drug but in the intensity of stimulus. Any way it can be deduced from the author's results that increase in intensity of stimulus, when concentrations of the drug and other factors remain the same, delays the drug actions. Hence the author assumes that the changes produced by an electrical stimulus in a nerve trunk, delay the action produced by the local anaesthetics either by preventing or setting up a process of an opposite nature to that of reversible adsorption equilibrium as produced by the local anaesthetics in nerve trunk, or at least these facts are evidence regarding the nature of conduction of an electrical impulse in a nerve trunk.

The author has already mentioned his inability to give a satisfactory explanation of the time x action curves of cocaine in rabbit's cornea as represented by the formula $(C - C_m)(T - T_m) = K$.

A partial explanation can, however, be given on certain probable assumptions. It is known that adsorption is usually a rapid process (Clark 1933 f) Hence the author assumes that in high concentrations it would be almost instantaneous, and rapid in lower concentrations. The second assumption is that there would be a minimum time for a single molecule of a drug to penetrate a membrane and an increase of the concentration of the drug will not decrease the time of penetration of the drug. The third assumption is that the time of penetration will be affected by concentrations at higher dilutions.

On these three probables the curve can be explained if it be divided in three portions: (a) a first portion where time of action remains practically constant and not much affected by increase in the concentration of the drug. It is this portion that corresponds to the minimum time of molecular penetration; (b) a second exponential part which mainly represents the rate of penetration of drug to changes in concentration, The rate of action of the drug after penetration of the drug being very rapid; (c) the third portion left over being a combination of the rate of penetration and rate of adsorption which in lower dilution may take some time to complete.

VI. SUMMARY.

1. It is impossible to obtain a satisfactory quantitative comparison of the local anaesthetic properties of two drugs by measurement of the rate of paralysis of the frog's sciatic nerve.

In the first place the preparation shows a very wide individual variation and in the second place the curve relating concentration and rate of action obtained with different drugs frequently cross so that different ratios are obtained with every concentration studied.

2. The rate of onset of anaesthesia in the rabbit's cornea is unsuitable as a measure of local anaesthetic potency for the same reasons as those given in the case of the frog's sciatic nerve.

3. The duration of action produced by local anaesthetics on the rabbit's cornea and on the human wheal are possible methods for quantitative measurements.

4. /

4. The cornea of different rabbits show considerable individual variation, but this can be eliminated by making repeated experiments on a single rabbit.

5. There is an approximate linear relation between the logarithm of the concentration of a local anaesthetic and the duration of action produced on both the rabbit's cornea and on the human wheal.

6. The relation between concentration and time of action can be expressed by the formula $C^n = K.T.$ and n usually has a value between 0.5 and 0.9.

7. Since there is no simple linear relation between time and concentration it is not possible to estimate differences in local anaesthetic potency by measuring differences in the duration of action. i.e. The fact that drug A produces an action of twice the duration of that produced by drug B at the same concentration does not imply that A is twice as potent as B.

8. The simplest method for comparing the relative potency of local anaesthetics is to measure the concentration/

concentration which produces local anaesthesia for a standard time, e.g. 10 min. This method can be employed either with the rabbit's cornea or the wheal method, but in the former case care must be taken to exclude errors due to individual variation.

9. The addition of adrenaline to local anaesthetics may change the ratio of activity as determined by the wheal method.

VII. REFERENCES.

Bovet, D. 1931. Arch. int. Pharmacodyn. 41, 103.

Clark, A.J. 1933a. Mode of Action of Drugs on Cells, E. Arnold. London. p. 74.

1933b. Ibid. p. 198.

1933c. Ibid. p. 4.

1933d. Ibid. pp. 43, 52, 151, 154,201.

1933e. Ibid. p. 124.

1933f. Ibid. pp. 67, 71.

Gessner, O., Klenke,J. and Wurbs, F.R. 1932. Arch. exp. Path. Pharmak. 168, 447.

Hill, A.V. 1928. Proc. Roy. Soc. B.104, 39.

Laubender, W. and Ost, W. 1932. Arch. exp. Path. Pharmak. 165, 520.

Regnier, J. 1923. C.R. Acad. Sci. Paris, 177,558; 1923. Bull. Sc. Pharm. 30, 580.

Rider, T.H. 1930. J. Pharmacol. Baltimore, 39,329.

Sollmann, J.E. 1914. Beitr. Klin. Chir. 91, 489. 1918. J. Pharmacol. Baltimore, 11,17.

Uhlmann, F. 1930. Arch. int. Pharmacodyn. 36,253.

PART II.

CHEMICAL CONSTITUTION AND PHARMACOLOGICAL ACTION.

I. CHEMICAL CONSTITUTION AND PHARMACOLOGICAL ACTION.

I. DESCRIPTION OF DRUG GROUPS TESTED.

The various drugs tested by the author and included in the text can be divided for the purposes of discussion under the following three groups:

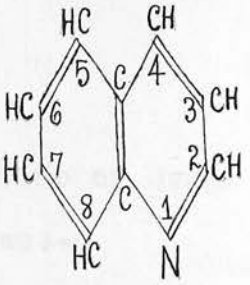
(a) Quinoline group.
(b) Pyrazoline group.
(c) Various other compounds.

The chief rings in these groups are shown in Fig. 9.

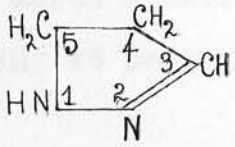
The structural formulae of these drugs are shown in appendix (Table A). Their chemical names are so cumbersome that it is necessary to describe them by identification numbers, and the key to these numbers is shown in the appendix (Table B).

Fig. 9 /

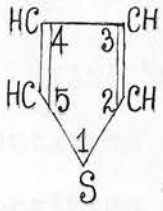
Fig. 9.



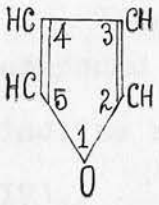
Quinoline nucleus.



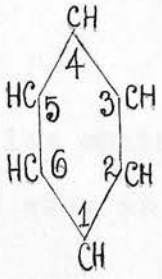
Pyrazole nucleus.



Thienes nucleus.



Furyl nucleus



Phenyl nucleus

The main group of drugs can be divided into sub-groups as follows:-

A. Quinoline Group.

(i) Compounds in which a carbon chain is connected to the quinoline ring at position 2 of the ring (Drug I, II).

(ii) Compounds in which a carbon chain is linked to the quinoline ring at position 2 by a nitrogen atom (Drug III, IV, V, VI).

(iii) This is a compound which has its chain attached to the quinoline ring by a C atom at position 6 (Drug XIV).

(iv) These are compounds in which a carbon chain is attached to the quinoline ring at position 8 (Drug XV, XVII, $XVIII_{1,2,3,8}$). These can be further subdivided into (a) monoquinolines (Drug XV, $XVIII_1$) and also the group i and iii; (b) Di-quinolines as drug XVII, $XVIII_2$, $XVIII_3$.

B. Pyrazoline Group.

This group of drugs consists mainly of substituted products/

products of pyrazoline compounds, the replacements being chiefly made at positions 1 and 5 of the pyrazoline ring. According to the radicles substituted they are divided into 5 sub-groups as follows:-

(a) (1) Furyl-phenyl group. Drug XVI_1, XVI_9
(2) Diphenyl group. " XVI_2, XVI_{10}
(3) Substituted phenyl group. " XVI_3 to XVI_8 and XVI_{11} to XVI_{16}

(b) (1) Piperidine pyrazoline. Drug XVI_1 to XVI_8, XVI_{11}, XVI_{13}, XVI_{14}

(2) Dimethylamine " Drug XVI_9, XVI_{10}, XVI_{12}, XVI_{15}.

C. Various Other Groups.

This group consists of three different series of compounds as follows:-

(1) Thienes Drug VII, VIII.

(2) Furyls " IX, X, XI.

(3) Amino-phenyls " XII, XIII, $XVIII_4$.

II. /

II. RELATION OF DRUG GROUPS AND PHARMACOLOGICAL ACTION.

(a) Potency.

The local anaesthetic potency of the drugs under consideration was compared by determining the concentration required to produce local anaesthesia for from 5 to 10 minutes, either in the rabbit's cornea or in the human wheal. Local anaesthetic effect that lasted for less than 5 minutes has been termed incomplete anaesthesia.

The results obtained with the 37 odd drugs tested are given in appendix, Table B. The tables given in the text (Table VI and Table VII) show results abstracted from the main table in the appendix.

Table VI. /

Table VI.

Drug groups	Potency		Ratio to cocaine
	Wheal	Cornea	
Cocaine	0.05	0.25	1
(A) Quinolines:			
(1) CH_2 chain in position 2	0.05	0.25	1
(2) N linked in do.			
(a) with Cl without MeO	-	>5.0–5.0	0.05
(b) without Cl with MeO	-	5.0	0.05
(3) CH_2 chain in position 6	over 0.25	>5.0	<0.05
(4) CH_2 chain in position 8			
(a) monoquinolines	0.1–0.01	1–0.1	5.0
(b) diquinolines	0.007–0.005	0.1–0.04	10.0 20.0
(B) Pyrazolines.			
(a) (1) Furylphenyls	0.025	0.20–0.07	2
(2) Diphenyls	0.01	0.07	5
(3) Substituted phenyls	0.01–0.005	0.07–0.025	10–20
(b) (1) Piperidino-pyrazolines	0.025–0.005	0.20–0.025	10
(2) Dimethylamine pyrazolines	0.025–0.01	0.2–0.05	5
(C) Various Other Compounds			
(1) Thienes	0.05	>5.0–0.25	1
(2) Furyls	0.10–0.05	0.5–0.25	1
(3) Aminophenyls	0.05	>5.0–0.25	1

The data given in Table VI show the following points.

(a) The activity of members of the sub-group A.2 in which the side chain is linked to the quinoline group by a nitrogen atom is less potent than that of sub-group A.1 in which the side chain is linked to the quinoline group by a carbon atom.

(b) Compounds with the side chain in position 6 (sub-group A.3) have a low local anaesthetic potency.

(c) Compounds with the side chain in position 8 (sub-group A.4) have a higher local anaesthetic potency than have compounds with the side chain in position 2 (sub-group A.1).

(d) Diquinoline compounds (sub-group A.4 b) have a higher potency than monoquinoline compounds (sub-group A.4a, 1, 2 and 3).

Another point to be noted is that, when the same radicle (methyl piperidine) when connected to the quinoline ring at position 6 is shifted to position 8, there is a marked increase in anaesthetic potency, particularly as regards the power of mucous membrane penetration (Drug XIV and XV, Table A and B, appendix).

B. /

B. Pyrazoline Group.

Sixteen pyrazoline compounds have been tested and all of them are powerful local anaesthetics. They are somewhat more potent than the quinolines particularly as regards their action on mucous membranes. Their potency is fairly level but as a general rule it is seen that the sub-group B.a.1. furyl phenyl are least potent. Next in order of potency comes the group B.a.2, Diphenyl ones which are more potent, and lastly the group B.a.3. substituted phenyl ones, which are most potent. Also the piperidino compounds (group B.b.1. show a higher potency than do corresponding dimethyl compounds (group B.b.2).

C. Various Other Compounds.

As a rule this group is fairly level in potency and has least power to penetrate mucosae as compared with group A and B. Of its various sub-groups it can be said that (a) aminophenyls (group C.2) are more potent than thienes (group C.1) while the furyls (group C.2) are the least potent.

From/

From drug $XVIII_1$ and $XVIII_4$ (Table B, appendix, Group A.4a and C.3) it can be inferred that substitution of aminophenyl by a quinoline ring markedly enhances anaesthetic potency. This was also found by Bovet (1931).

The general result of these tests was to indicate that the following compounds were most likely to prove of clinical service.

(1) Drug XVI/8 pyrazoline.

(2) " XVII diquinoline.

(3) " $XVIII_1$ monoquinoline.

(b) Irritation.

The practical value of a local anaesthetic depends upon its being non-irritant in the concentration in which it is employed. It is only possible to make a rough quantitative estimate of the irritant properties of drugs on the cornea and in the intra-dermal wheal, but such estimates are shown in Table B. (appendix). Within each of the groups the irritant action varies widely and a summary/

summary of the irritant properties of the sub-groups is shown in Table VII. A study of this table shows that:

(a) In group A (quinolines), these that have the side chain connected to the quinoline by carbon atom are less irritant than those that are connected by nitrogen atom.

(b) In group B (pyrazolines), substituted phenyls (group B.a.3) are least irritant.

(c) In group C of various compounds the amino-phenyls (group C.3) are least irritant as compared to furyls and thienes (group C.1 and 2).

Table VII. /

Table VII.

Drug Groups	Irritation	Ratio to cocaine	Toxicity	Ratio to cocaine
Cocaine	+	1	100	1
A. Quinolines				
(1) CH_2 chain in position 2	+	1	-	-
(2) N linked in position 2.				
(a) with Cl without MeO	+++ to ++++	4	-	-
(b) without Cl without MeO	do.	4	-	-
(3) CH_2 chain in position 6	+++	3	-	-
(4) CH_2 chain in position 8.				
(a) monoquinolines	<+ to ++	1	50- >70	0.6
(b) diquinolines	<+ to +	0.5	<10-90	0.5
B. Pyrazolines.				
(a)(1) Furyl phenyls	++	2	50- >120	0.9
(2) Diphenyls	+++ to ++++	4	50-100	0.7
(3) Substituted Phenyls	<+ to +++	1	<25-100	0.5
(b)(1) Piperidino-pyrazolines	<+ to ++++	2	<25-90	0.6
(2) Dimethylamine pyrazolines	++ to +++	3	50->120	0.8

C./

Table VII. contd.

Drug Groups	Irritation	Ratio to cocaine	Toxicity	Ratio to cocaine
C. Various other groups.				
(1) Thienes	<+ to ++++	2	<25	0.2
(2) Furyls	++++	4	50-150	1.0
(3)Benzamides (or aminophenyls)	<+ to ++	1	<25-500	3.0

(c) Toxicity.

The toxicity of the drugs was measured by intravenous injection into mice. A general estimate of the toxicity of all drugs with a well marked local anaesthetic action was made by finding the approximate lethal dose and then using 6 mice to estimate the median lethal dose. In the case of the most promising drugs the median lethal dose was estimated accurately by using 50-100 mice. The results are shown in detail in Table B (appendix) and are summarised in Table VII.

In general it was found that the toxicity of the drugs within each sub-group varied widely, but that the range of toxicity was similar in all the sub-groups tested. The toxicity measurements do not therefore show any facts of outstanding importance regarding the relation between pharmacological action and chemical constitution. These measurements are however essential for the estimate of the clinical value of the drugs in comparison to cocaine.

III. DISCUSSION.

(a) Quinoline Group.

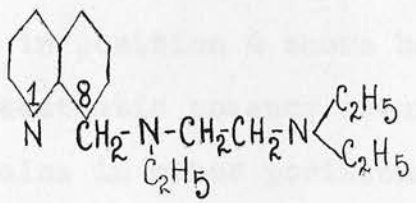
The author's results show that the local anaesthetic potency of these compounds varies as follows:-

Position of side chain	Element in side chain proximal to the ring	Local anaesthetic potency as compared to cocaine
position 6	C	< 0.05
position 2	N	0.05
position 2	C	1.0
position 8	C	5.0-10.0

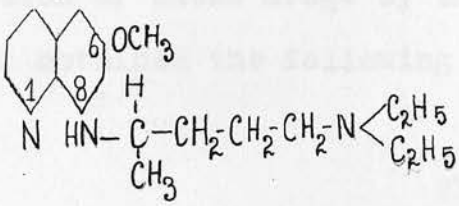
The influence of the position of the side chain on pharmacological potency is in accordance with the findings of other authors. Two other important quinoline derivatives are of the same type, namely plasmoquine and Drug 665 (Fourneau). The formulae of these drugs is shown in Fig. X.

Fig. X./

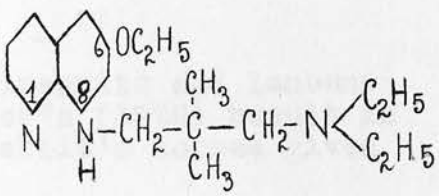
Fig. X.



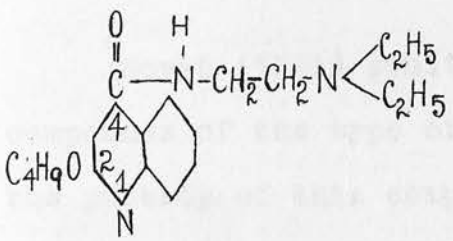
Author's drug $XVIII_2$



Plasmoquine



Fourneau and Bovet's compound 665



Nupercaine

The fact that in nupercaine the side chain is in position 4 shows however that local anaesthetic potency occurs in compounds with side chains in other positions.

Bovet (1931) studied the local anaesthetic action of these drugs by the rabbit's cornea method and obtained the following results.

	Plasmoquine = 8 x cocaine
	Drug 665 = 58 x cocaine
	Nupercaine = 53 x cocaine
Lipschitz and Lauben-der's (1929) result in rabbit's cornea gives	Nupercaine = 100 x cocaine
Author's result gives	Drug XVIII$_i$ = 5 x cocaine

Bovet (1931) studied a long series of compounds of the type of drug 665 and showed that the potency of this compound was dependent on the group in position 6. When this group was absent the compound had scarcely any local anaesthetic action and the substitution of an ethoxy for a methoxy group in position 6 increased the local anaesthetic/

anaesthetic potency about sevenfold.

The fact that nupercaine also has a butoxy group attached to the quinoline ring (in this case in position 2) indicates the importance of this group.

Fig. 10 shows that drug 665 the side chain is united to the ring by a nitrogen (N) atom. In the substances studied by the author there was no group at position 6 (Drug IV, V, XIV are weak) and in this case the compounds with a proximal N atom in the side chain had very little local anaesthetic action, a result which agrees with Bovet's findings.

On the other hand when the side chain was united to the ring by a carbon atom (Drug $XVIII_i$) the compound had a powerful local anaesthetic action. These results suggest that a drug of the type $XVIII_i$ with the addition of an ethoxy group at position 6 would be a very potent local anaesthetic. Unfortunately the technical difficulties of constructing a compound of this type are at present insuperable.

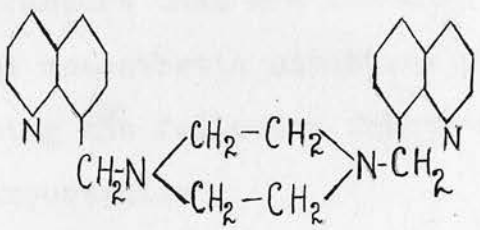
The author's measurements of toxicity showed that compounds in which two quinoline groups are linked/

linked to the same N atom are more toxic than when two groups are linked to different N atoms or compounds in which one ring is united to a straight chain.

The author's results also show that duplication of a single local anaesthetic structure enhances local anaesthetic potency. (1) The diquinolines are more potent than mono-quinolines; (2) Amongst the mono-quinolines those having two radicles of diethylamine are more potent than those having only one radicle of diethylamine; (3) also the diphenyls in the pyrazoline are more potent than mono or furylphenyls. Hence the author concludes that duplication of local anaesthetic structure is liable to enhance potency.

It must be seen from the results that this duplication of anaesthetic structure to enhance potency might not necessarily be favourable for other conditions required for an ideal local anaesthetic, such as toxicity and irritation. As the results in Table VII shows that diphenyls are more irritant than furylphenyls and diquinolines more toxic (except one XVII) than monoquinolines. Hence/

Hence the author concludes from the results that such alterations are not desirable if not compensated by adequate changes in conditions of arrangement. Such as in the present case (1) diquinolines should be linked by carbon atom to the quinoline ring to reduce irritation and to increase potency, or (2) nitrogen chains should be reduced to closed chains. However the best result of the quinolines as obtained by the author is the diquinoline drug XVII having the formula:-



It has a low toxicity about equal to cocaine and potency about ten times of cocaine.

(b) Pyrazoline Compounds.

The results show that furylphenyls (group B.a.1) are weaker than the diphenyls, hence the author concludes that the phenyl radicle is essential for pyrazolines, possibly at position 1 and 5.

Extensive substitution in the phenyl groups (e.g. addition of methoxy radicles etc.) produces relatively/

relatively less effect in potency or toxicity but reduces irritation considerably. On the other hand the piperidine compounds show a higher toxicity than the diaminomethyl ones. Hence the author infers that the factor of irritation to be somehow related to the phenyl radicle, and the factor of reduction in toxicity to be related to the dimethylamine radicle respectively in the synthesis of the pyrazolines.

The author in his discussion has tried to find out factors that are involved in the synthesis of a local anaesthetic structure and has succeeded in eliciting the following three, all of which are equally important:-

(a) That all potent local anaesthetic compounds must consist of one or more local anaesthetic structure.

(b) That these structures must be placed or linked at favourable positions of the anaesthetic structure.

(c) That alterations in the conditions of arrangements (as referred to in text) are necessary for a further increase in potency.

The author has attempted to fit the above conclusions/

conclusions to the present synthetic compounds as follows.

The pyrazoline compounds of the present series have a common anaesthetic structure like the pyrazole compound antipyrine, i.e. the pyrazole nucleus. Anaesthetic potency of the pyrazolines is 100 x antipyrene. This marked change in potency is explained as due to alterations in the conditions of arrangements at position 3 and 5 of the pyrazole nucleus (i.e. substitution of piperidine and phenyl respectively).

IV. /

IV. INFERENCES AND DEDUCTIONS.

The author in his results, as already mentioned, obtained a marked increase in potency when an aminophenyl radicle was replaced by a quinoline radicle in compounds $XVIII_4$ and $XVIII_1$. From this result he deduces that it is highly probable that the aminophenyl radicle in novocaine if replaced by a quinoline radicle may enhance its potency considerably, and as quinoline compounds as a rule are more efficient in penetrating the mucosa than its corresponding amino-benzoates, this hypothetical compound is expected to be more potent in the cornea than novocaine.

Some of the previous results of the author show that duplicating anaesthetic structures enhances anaesthetic effect. The phenyl in 5-position of the pyrazoline compound is easily replaceable and the author infers that if it is replaced by a quinoline radicle there is a fair chance of the hypothetical quinoline pyrazoline compound to be potent. Besides as the substitution of/

of phenyl radicle by a quinoline as in the present case is unlikely to give very different results.

From the evidence of the quinoline compounds as obtained by the author, as well as those of other workers, the following results are obtained as requirements for an ideal quinoline local anaesthetic.

(1) The nitrogen of the chain radicle should be indirectly connected to the ring of the quinoline radicle.

(2) The position of linking the chain to the ring should be either at 8 or 4 position of the quinoline.

(3) There should be an additional chain attached to other positions of quinoline ring preferably at 6 position or 2 position.

(4) It should have two quinoline rings preferably.

(5) The quinolines should be linked to different N atoms.

(6) The nitrogen atoms of the chain should be linked as close chains, as opposed to open chains.

(7) The carbon atoms of the chain be preferably linked as crossed chains than straight chains.

It/

It will be seen that drug XVII and nupercaine fulfils most of the conditions of an ideal quinoline compound. Both have the same therapeutic ratio for its base in the human wheal. Drug XVII is less toxic and less potent, while nupercaine is more toxic and more potent. Hence the author deduces from above that both compounds have room for improvement: (a) nupercaine can be converted to a diquinoline compound and its two nitrogen atoms should be linked as close chains, (b) Drug XVII should have a chain radicle attached to each of its quinoline at position 6 or 2.

V. /

V. SUMMARY.

1. It has been proved that pyrazoline nucleus is a potent anaesthetic structure.*

2. Structural conditions and other factors that go to make an ideal quinoline local anaesthetic have been investigated and defined and certain hypothetical compounds, likely to be of anaesthetic value, have been suggested, by improving the already known local anaesthetics such as novocaine and nupercaine.

3. Of the various essentials for obtaining a local anaesthetic compound the following three have been elicited from the results:

(a) presence of a local anaesthetic structure as quinoline, pyrazolines etc.

(b) a local anaesthetic position in relation to the local anaesthetic structure where other structures are to be linked.

(c) other alterations in structural arrangement as described in the text, i.e. linking of additional side chains in the quinoline ring.

4. The relation of toxicity and irritation to chemical constitution has been discussed.

* The first of the series of the pyrazoline drugs was also found out by Professor A.J. Clark previous to the author who tested the subsequent number of the series.

References.

Bovet, D. 1931. Arch. int. Pharmacodyn. 41, 103.

Fourneau, E.M., Trefouel, 1931. Ann. Inst. Pasteur.
Bovet, D, and Benoit,G. 46, 514.

Lipchitz and Laubender. (1929) Klin. Wschr. 2, 1438.

PART III.

I. /

Special Test of Most Important Drugs.

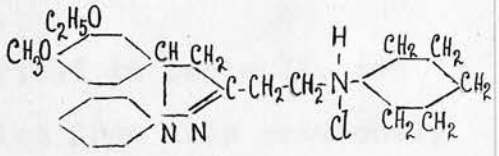
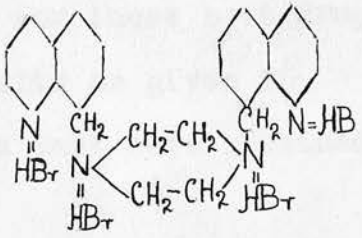
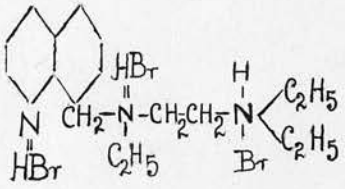
Special detailed tests were made upon the three best drugs obtained with the object of finding out how far they might be of clinical value. The three drugs tested were the following:

(1) Drug XVI/8 .... Pyrazoline piperidine

(2) Drug XVII .... Diquinoline.

(3) Drug XVIII/1 ... Monoquinoline.

Their chemical formulae are given below.

Drug No.	Chemical formula	Structural formula.
XVI/8	Monohydrochloride of 1-phenyl-3(β-N-piperidino ethyl) 5-(3'-ethoxyl-4-methoxyl) phenyl pyrazoline.	
XVII	Tetra-hydrobromide of N and N-piperizino-8-methyl quinoline and 8-methyl quinoline.	
XVIII/1	Trihydrobromide of 8-(diethylamino-ethyl) ethylamino methyl) quinoline	

These drugs are all soluble in water, and stable when boiled, but are not antiseptics.

The detailed tests made regarding their potency as compared with cocaine in rabbit's cornea, human wheal and frog's sciatic, have already been described in Part I. The method of comparing toxicity was done with 50-100 odd mice by Trevan's technique as has already been described in Part II, and the details of the technique are given in the appendix. The comparison of irritation was only approximate.

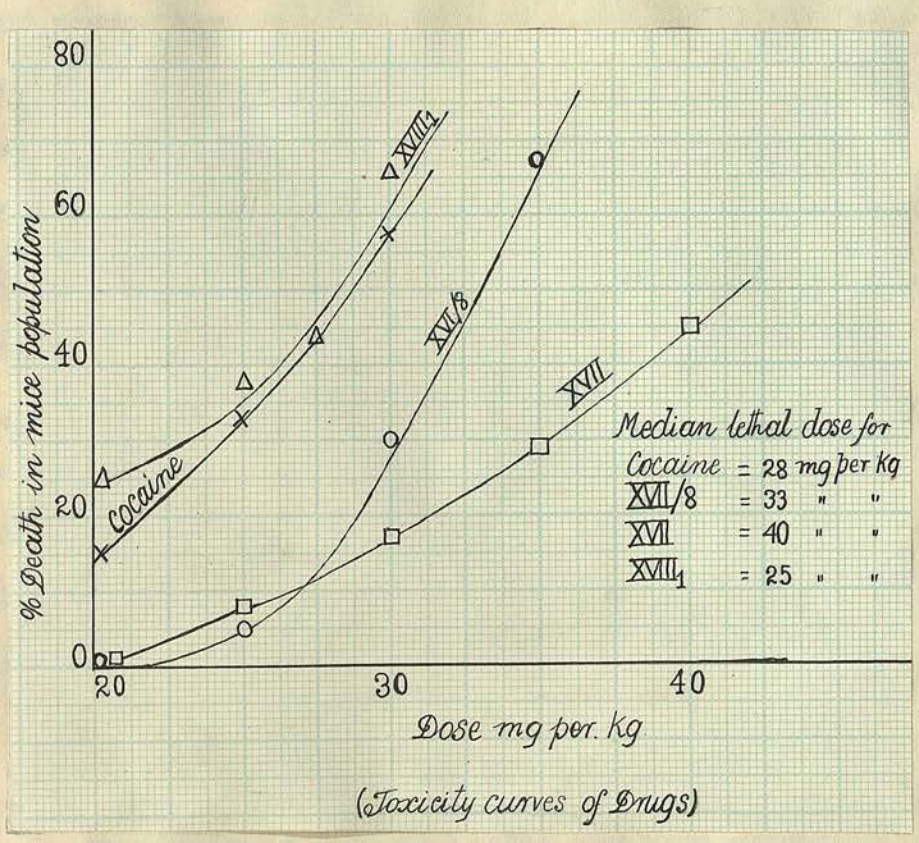
Results.

The results are summarised in Table IX. and Fig. 11. Table IX is compiled from data previously obtained in Part I from Figs. 7 and 8 and Table I which gives Log x Concentration curves of tests on rabbit's cornea and human wheal for these best drugs and from the frog's sciatic results as given in Table I, Part I, or from results that were obtained by the same method.

Table IX /

Table IX.

Method of tests	Comparative potency to cocaine.			
	Cocaine	XVI/8	XVII	XVIII/1
Frog's Sciatic.				
(a) Percentage of drug with equal time of onset of paralysis as cocaine	1	<1 >0.5	<2 >1	<2.5 >1.5
(b) Ratio to cocaine	1	1.5	0.75	0.5
Rabbit's Cornea.				
(a) Percentage of drug giving anaesthesia for 10 min.	0.32	0.02	0.045	0.09
(b) Ratio to cocaine	1	16	7	3.5
Human Wheal.				
(a) Percentage of drug producing anaesthesia for 10 min.	0.1	0.008	0.026	0.036
(b) Ratio to cocaine	1	12	4.5	2.75
Irritation.	+	+	<+	++
Toxicity to mice.				
(a) Median lethal dose	28	33	40	25
(b) Ratio to cocaine	1	1	0.75	1



(Toxicity curves of Drugs)

Fig. 11.

The median lethal dose (i.e. the dose giving 50% mortality when Trevan's method is used) for mice was obtained from Table C (appendix) and the results plotted as in Fig. 11, show the relation between dose and mortality in population. The abscissa gives dose of various drugs calculated to mg. per kg. body weight and the ordinate gives mortality per cent. of mice population at various doses of each drug. The dose that gives 50% mortality can be then read off.

The results give a high therapeutic index for each drug, hence it was desired to do a final test on human fingers (i.e. digital nerve trunks) with adrenaline, with novocaine as control. These tests were carried out as follows:-

Anaesthesia of Human Digital Nerve.

This method does not give very accurate quantitative results, but has the advantage of measuring the activity of drugs under conditions closely resembling those under which they are used clinically. The importance of obtaining fairly direct evidence regarding the clinical efficiency of local anaesthetics has already been pointed out.

Method/

Method.

0.5 c.c. of a known percentage of the drug at pH 6-7 was sterilised by boiling in isotonic saline and adrenaline. 0.002% was added. The solution was injected at the root of the fingers as near as possible to the digital branch of the radial nerve as is illustrated in Fig. 12. Anaesthesia was deemed to be complete if the sensation to pin-prick was abolished in the area of the distribution of the fibres of the nerve trunk distal to the point of injection, i.e. up to the root of the nail on the lateral half of the finger injected.

Results.

The summary of results is given in Table X from details of the protocol (Table D in appendix). It shows that only XVIII/1 is good for giving deep anaesthesia in nerve conduction as compared to novocaine, and that drug XVI/8 is worst of all, while drug XVII is comparatively better than drug XVI/8, and that in concentrations when it equals novocaine, it shows a favourable margin of therapeutic ratio as compared to novocaine. However it is not so reliable for the production of deep anaesthesia as drug XVIII/1.

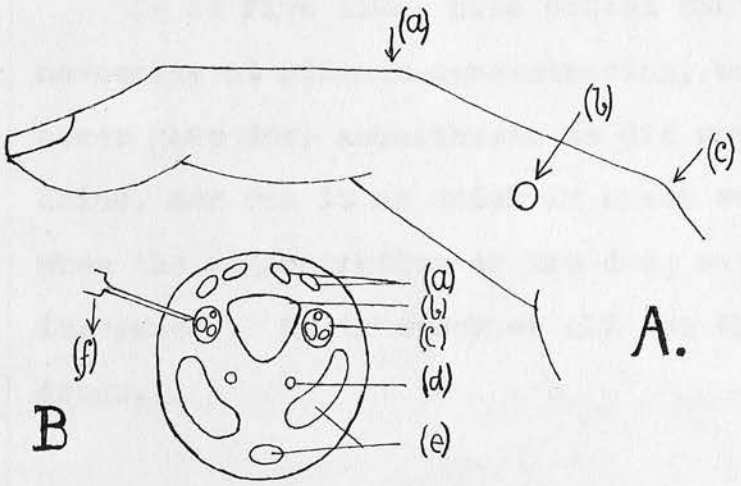


Fig. 12. Diagram showing site of injection in human digital nerve.

A. Dorso-lateral view.
(a) 1st and 2nd phalengeal joint.
(b) Site of injection.
(c) Metacarpo-phalengeal joint.

B. Cross-section view of A.
(a) Extensor tendons.
(b) 1st phalanx.
(c) Sheath of dorsal branches of radial nerve and vessels
(d) Ventral branches of median nerve and vessels.
(e) Flexor muscles and tendons.
(f) Course of the needle.

Table X.

Summary of the data of human digital nerve experiments from protocol (Table D, appendix).

Drug no.	Results.
XVI/8	It is five times more potent than novocaine at minimum concentration, but never gave deep anaesthesia as did novocaine, nor was it as quick in onset even when the concentration of the drug was increased. It is worst of all the three drugs.
XVII.	It requires a higher concentration than drug XVI/8 for effecting anaesthesia. At still higher concentration it gives deep anaesthesia. Its onset time of anaesthesia is longer than novocaine. It is 2.5 times as potent as novocaine. It is better than drug XVI/8.
XVIII/1.	It is equal to novocaine in its quick onset, and produces as deep anaesthesia. It is best of all.

Summary and Conclusion.

1. That the drugs XVI/8 and XVII, i.e. the pyrazoline and the diquinoline respectively, are good for clinical application in mucosae and infiltration anaesthesia, but they cannot replace novocaine in nerve trunk anaesthesia.

2. Drug XVII/1 is just equal to novocaine in nerve trunk anaesthesia, and hence in absence of a wide margin of its therapeutic index from that of novocaine, it will have very little chance to replace novocaine. Synthetic series of this compound as opposed to the other compounds may yield better results.

3. Drug XVII (diquinoline) has a better chance to replace novocaine than Drug XVIII/1 for human nerve trunk anaesthesia, and is an all round drug for clinical use.

Acknowledgments.

I gratefully acknowledge the advice of Professor A.J. Clark throughout the course of my work. I thank Dr W.O. Kermack of the Laboratory of the Royal College of Physicians for the detailed advice about the chemical portions of the work.

My thanks are also due to Drs. W.O. Kermack, T.W. Wight and W. Muir for supplying me with the synthetic quinolines, and to Dr H. Nisbet for supplying me with the synthetic quinolines/α Pyrazolines which I have investigated.

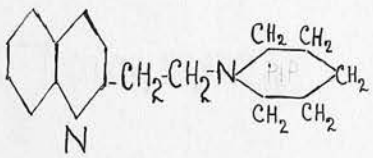
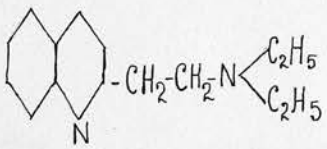
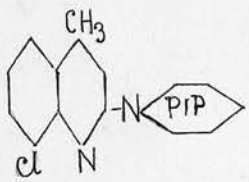
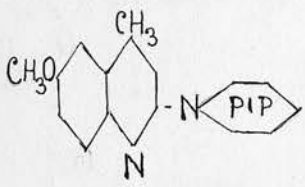
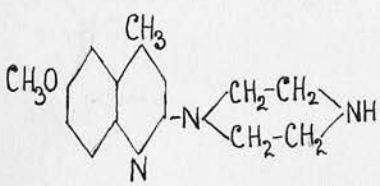
The expenses of this investigation were paid by the Moray Fund of the University of Edinburgh.

APPENDIX.

I. Table A.

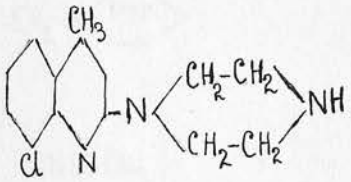
List of Synthetic Drugs Investigated.

First Series.

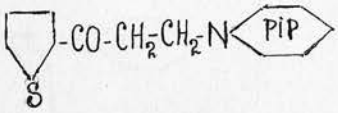
No*	Chemical Formula	Structural Formula
I.	2.β-piperidino ethyl quinoline	
II.	2.β-diethylamino-ethyl-quinoline	
III.	2-N.piperidino-4-methyl-8-chloro-quinoline	
IV.	2-N.piperidino 4-methyl-6-methoxy quinoline.	
V.	2-piperizino 4 methyl-6-methoxy-8-chloro-quinoline.	

* Compounds numbered as XVI_1 are the same as when numbered XVI/1.

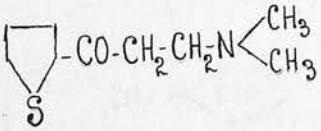
VI. 2-piperazino-4-methyl-8-chloro-quinoline.



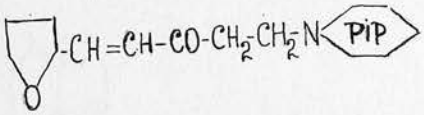
VII. 1-N-piperidino 3-(2-thiene) propaine 3-one



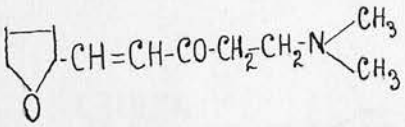
VIII. 1-dimethyl-amino-3(2-thiene propaine) 3-one



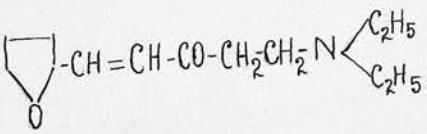
IX. 1-N-piperidino 5-furyl-4-pentene 3-one



X. 1-dimethylamino-5-furyl-4-pentene 3-one.

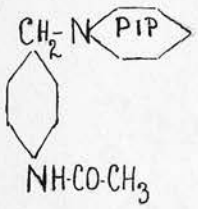


XI. 1-diethylamino-5-furyl-4-pentene 3-one.

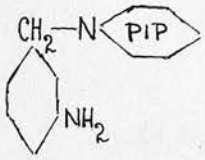


XII /

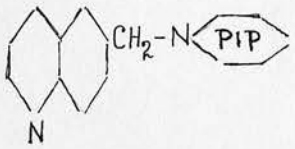
XII. p-acetyl-amino piperidino methyl benzene



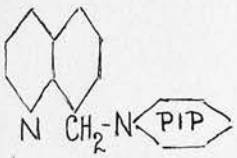
XIII. o-amino-piperidino methhyl benzene



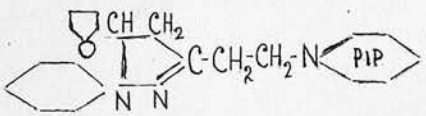
XIV. 6-piperidino methyl-quinoline.



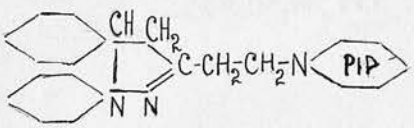
XV. 8-piperidino methyl-quinoline.



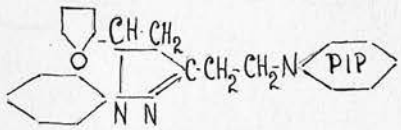
XVI, 1-phenyl-3(β-piperidino-ethyl) -5-furyl - pyrazoline.



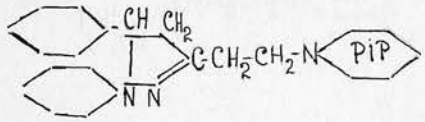
XVI2 1-5-diphenyl 3-N-piperidino ethyl pyrazoline.



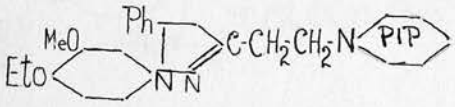
XVI/1. 1-phenyl-3(β-piperidino ethyl)5-furyl pyrazoline.



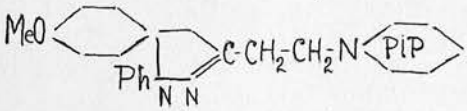
XVI/2. 1:5-phenyl 3(β-piperidino ethyl) pyrazoline.



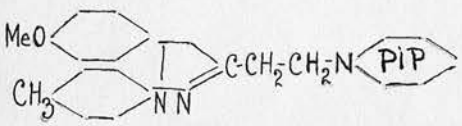
XVI/3. 1-(3'-methoxy 4'-ethoxyphenyl 3(β-piperidino ethyl)5-phenyl pyrazoline.



XVI/4. 5-(4'-methoxy phenyl-3(β-piperidino ethyl) 1-phenyl pyrazoline.

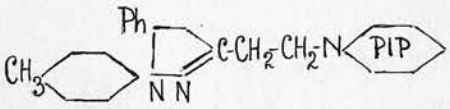


XVI/5. 1-p-tolyl-3-(β-piperidino ethyl-5-p-methoxyphenyl pyrazoline.

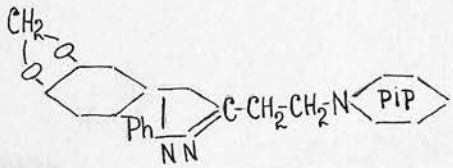


XVI/6 /

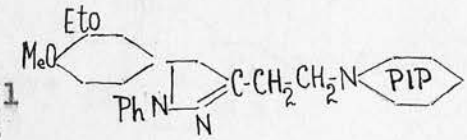
XVI/6. 1-p-tolyl 3(β-piperidino ethyl) 5-phenyl pyrazoline.



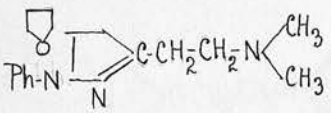
XVI/7. 1-phenyl-3(β-N-piperidino ethyl-5(3'-4' methylene diozyohenyl pyrazoline.



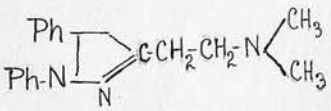
XVI/8. 1-phenyl-3(β-N-piperidino ethyl)5(3'-ethoxyl 4'-methoxylphenyl pyrazoline.



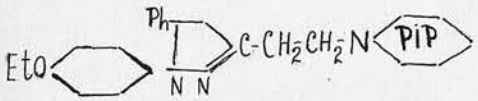
XVI/9. 1-phenyl-3(β-dimethylamino-ethyl)5-furyl pyrazoline.



XVI/10. 1:5-diphenyl-3(β-dimethylamino ethyl)pyrazoline.

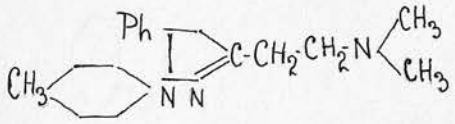


XVI/11. 1-p-ethoxyphenyl-3(β-piperidino ethyl)5-phenyl pyrazoline.



XVI/12. /

XVI/12. 1-p-tolyl-3(β-dimethyl aminoethyl-5-phenyl pyrazoline.



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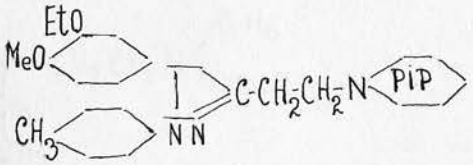

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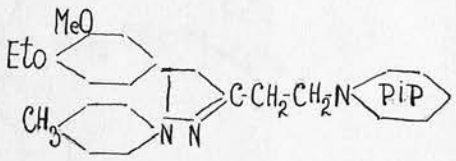
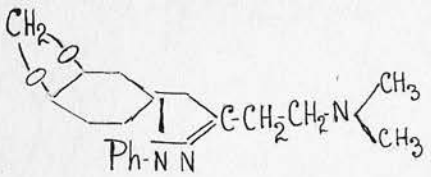
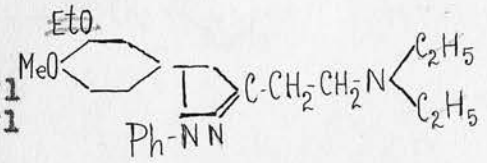


XVI/15. 1-phenyl-3(β-dimethylamino-ethyl)5(3'-4'-methylenedioxy-phenyl pyrazoline.

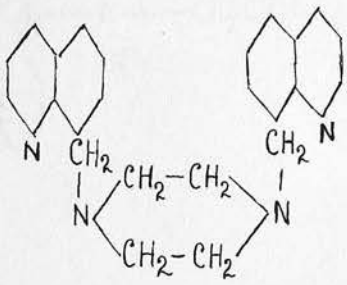


XVI/16. 1-phenyl-3(β-diethylamino-ethyl 5(3'-ethoxyl 4'-methoxyl phenyl pyrazoline.



XVII/

XVII.
Bromide



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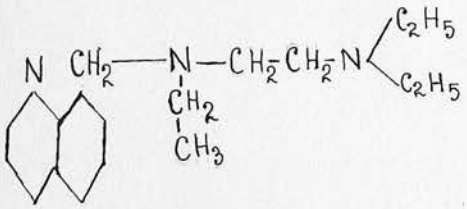

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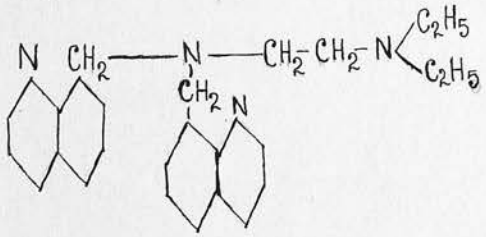

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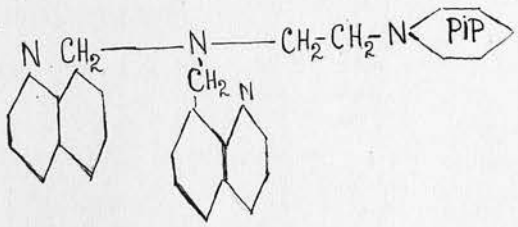

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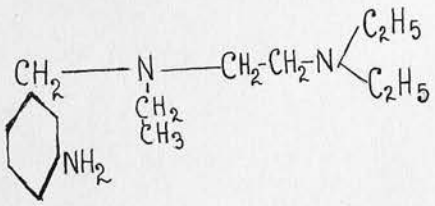
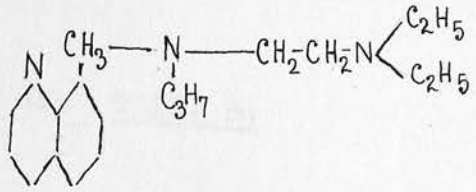


XVIII/6.



II. TABLE B.

Grouping of Drugs and Their Pharmacological Action.

A. Quinolines.

(1) Those which have $CH_2^{''}$ chain linked at position 2.

(2) Those which have N atom linked at position 2.

(a) with a Cl and without a MeO radicle in the ring.

(b) without Cl and with do. do.

(3) Those which have CH_2 chain linked at position 6.

(4) Do. do. do. 8.

(a) Monoquinolines.

(b) Diquinolines.

B. Pyrazolines

(a) (1) Furylphenyls, pyrazolines.
(2) Diphenyls. do.
(3) Substituted phenyls, do.

(b) (1) Piperidino-pyrazolines.
(2) Dimethylamine do.

C. Various Other Groups.

(1) Thienes
(2) Furyls
(3) Benzamides

Table B.

Drug	Wheal	Cornea	Sciatic	M.L.D. i.v.i.	Irritation
Cocaine	0.05	0.25	1.0	100	+
A. Quinolines.					
(I) I.	0.05	0.25	1.5	-	+
II.	-	0.25	5.0		+
(2)(a) III.	-	5.0	0.5	-	+++
VI.	-	>5.0	<5.0	-	++++
(b) V.	-	5.0	0.6	-	++++
IV.	-	>5.0	<1.0	-	++++
(3) XIV.	>0.25	>5.0	5.0	-	+++
(4)(a) XV.	0.10	1.0	1.0	50	<+
XVIII/1	0.01	0.1	-	>70	++
XVIII/6	0.01	0.1	-	>70	++
(b) XVII.	0.007	0.1	-	90	<+
XVIII/21	0.005	0.05	-	10	+
XVIII/5	0.005	0.04	-	<10	+

Table B. contd.

Minimum Effective Concentration.

Drug	Wheal	Cornea	Sciatic	M.L.D	Irritation
Cocaine	0.05	0.25	1.0	100	+
B. Pyrazolines.					
(a)(1).					
XVI/1	0.025	0.2	0.2	50	++
XVI/9	0.025	0.07	0.2	>120	++
(2).					
XVI/2	0.01	0.07	0.2	50	++++
XVI/10	0.01	0.07	0.2	100	+++
(3).					
XVI/3	0.005	0.07	-	50	++
" /4	0.005	0.05	-	80	+
" /5	0.005	0.025	-	70	+++
" /6	0.005	0.025	-	90	+
" /7	0.005	0.025	-	60	+
" /8	0.005	0.025	0.7	70	+
" /11	0.005	0.03	-	<25	<+
" /12	0.01	0.05	0.4	50	++
" /13	0.01	0.025	-	<50	+
" /14	0.01	0.025	-	50	<++
" /15	0.01	0.05	-	100	++
" /16	0.005	0.025	-	70	++
(b)(1).					
XVI/1	0.025	0.2	0.02	50	++
" /2	0.01	0.07	0.02	50	++++
" /3	0.005	0.07	-	50	++
" /4	0.005	0.05	-	80	+
" /5	0.005	0.025	-	70	+++
" /6	0.005	0.025	-	90	+
" /7	0.005	0.025	-	60	+
" /8	0.005	0.025	0.7	70	+
" /11	0.005	0.03	-	<25	<+
" /13	0.01	0.025	-	<50	+
" /14	0.01	0.025	-	50	<+
" /16	0.005	0.025	-	70	++

Table B. contd.

Drug	Wheal	Cornea	Sciatic	M.L.D.	Irritation
(b)(2)					
XVI/9	0.025	0.02	0.2	>120	++
" /10	0.01	0.07	0.2	100	+++
" /12	0.01	0.05	0.4	50	++
" /15	0.01	0.05	-	100	++
6. Various Other Groups.					
1. VII.	0.05	0.25	0.5	< 25	< +
VIII.	-	>5.0	0.5	-	++++
2. IX.	0.05	0.25	>5.0	50	++++
X.	0.1	0.50	>5.0	150	++++
XI.	0.1	0.50	0.8	50	++++
3. XII.	-	>5.0	5.0	500	< +
XIII.	-	5.0	0.6	25	++
XVIII/4	0.05	0.25	-	-	++

Table C.

Detailed results of best drugs of the three structural types.

Data of median lethal dose.

Wt. of mouse about 20 gm.

Drug no.	c.c.of fluid injected	No. of mice injected	No.of mice killed	Mortality %
Saline control	1.0	15	0	0
Cocaine 0.1%	0.6	15	9	58
	0.5	15	5	33
	0.4	15	2	15
Novocaine 0.2%	0.9	15	7	50
	0.8	15	5	33
	0.6	15	1	7
XVI/8. 0·1%	0.8	6	4	66
	0.7	28	18	68
	0.6	32	10	30
	0.5	17	1	6
	0.4	16	0	0
XVII. 0·1%	0.8	33	16	45
	0.7	21	7	30
	0.6	15	3	18
	0.5	15	1	7
	0.4	2	0	10
XVIII/1. 0·1%	0.6	19	13	67
	0.55	26	11	43
	0.50	18	7	38
	0.40	12	3	25

Digital Nerve Anaesthesia (Human)

Novocaine%.	Drug%	Ratio of potency. Novocaine = 1.
0.5 (Subject K.M.) Complete anaesthesia up to the finger tip after 40-60 min. anaesthesia remained for 3½ hours. Later finger was tender.	XVI/8 0.1 Anaesthesia after 1 hour much less marked than novocaine. It did not reach the finger tip but remained longer than novocaine though injected earlier.	5
0.5 (Subject K.M.) Anaesthesia took 20-40 min. to complete. It disappeared in 3 hours. It did not reach the finger tip. There was irritation in finger later.	0.2 Anaesthesia after 20 min. It was not as deep as novocaine, but lasted longer. It disappeared in 3-4 hours. There was no irritation later.	3
1.0 (Subject K.M.) Anaesthesia was complete in 15 min. near to but not to the finger tip. It disappeared in 5 hours.	0.4 Anaesthesia was never deep to the extent as novocaine was but the anaesthesia though less extensive lasted for 7 hours.	3

(Subject K.M.) Complete anaesthesia in 15 min. It disappeared in 3 hours. There was no irritation later.	Complete anaesthesia in 30 min. but not to the finger tip. Anaesthesia was disappearing after 1 hour but remained for 5 hours.	> 3
(Subject C.K.) Anaesthesia was complete in 10 min. and lasted 2-3 hours.	Parasthesia remained locally. It was earlier to go than novocaine	< 2.5
(Subject C.M.S.) Complete anaesthesia up to the finger tip in 40 min. It disappeared in 3 hours.	Anaesthesia in 40 min, but not very deep and did not reach the finger tip but lasted for 5 hours.	3
-	Drug XVII. 0.1%	
(Subject K.M.)	No anaesthesia.	
0.5%	0.2%	
(Subject K.M.) Complete anaesthesia from 20-40 min, but did not reach the finger tip. It lasted for 3 hours. There was irritation later.	Complete anaesthesia in 10-20 min. but did not reach the tip. Anaesthesia lasted as long as novocaine did. There was no irritation later.	2.5
1.0% /		

1.0%	DrugXVII. 0.4%	
(Subject K.M.)		
Complete anaesthesia in 15 min. and it lasted for 5 hours.	Complete anaesthesia in 10-30 min., the deepest part was the tip. It lasted 15 min. more than novocaine. The finger was a little more tender than novocaine on pressure.	3
(Subject W.T.)		
Complete anaesthesia in 15 min. up to the finger tip which disappeared in 2½ hours. It pained later.	Complete anaesthesia but not to the finger tip. It lasted for 2½ hours.	3
(Subject D.T.)		
Complete anaesthesia in 20 min. but not to the finter tip. It lasted for 3 hours. There was slight irritation later.	Anaesthesia in 20 min. but not to the finger tip. It was less than novocaine.	<3
(Subject C.M.)		
Complete anaesthesia in 10 min. but not to the finger tip. It lasted for 3½ hours but was disappearing after 45 min.	Anaesthesia in 15 min. but not to the finger tip. It was passing away after 1½ hours but lasted for 7 hours.	5
(Subject H.K.)	Anaesthesia was complete in 10 min. but did not reach the tip. It was passing away in 2 hours but remained till 6 hours.	5

1.0%	Drug XVIII/1. 0.4%.	
(Subject H.K.)		
Complete anaesthesia in 10 min. It came up to the tip in 30 min. and lasted for 2 hours.	Complete and deep anaesthesia in 25 min. It came up to the tip in 50 min. and lasted for 2 hours.	2.5
(Subject K.M.)		
Both novocaine and drug XVIII/1 were very parallel in their onset and their disappearance. They gave deep anaesthesia in 15 min. which lasted for 2 hours.		2.5

III. Technique Employed in Testing New Drugs.

Certain special modifications of technique were used in this investigation. In the first place it was desired to get quantitative estimates of the local anaesthetic activity of a large number of compounds in order to obtain information regarding the relation between chemical constitution and pharmacological action. This involved testing many drugs with low solubility or markedly irritant action even though they were obviously unsuitable for clinical use, and here it was necessary to use not only a standard technique, but also modification of this technique. Secondly an attempt was made to devise a method of quantitative estimation which would give a direct indication of the clinical value of certain selected drugs.

(a) /

(a) Anaesthesia of Rabbit's Cornea.

In this method a few drops of fluid are introduced into the conjunctival sac and kept there for a varying period. The most obvious source of error is the dilution of the fluid by tears and the loss of fluid through the lachrymal duct. It is therefore difficult to maintain a fixed concentration of fluid in the eye.

The technique devised by Sollmann and modified by Rider (1930) has already been described and was adopted by the writer. In this method the eye is irrigated for a minute. This is suitable for testing powerful local anaesthetics but unsuitable for testing drugs with low solubility and low anaesthetic activity. In such cases the fluid was kept in contact with the cornea for five minutes and then the sensitivity of the cornea was measured. If the result was negative, the eye was flooded for a second, and if necessary, for a third period of five minutes. A negative result therefore indicated that a solution did not produce anaesthesia so as to last for more than 5-10 minutes after being in contact with the cornea for 15 minutes. Some writers/

writers have recommended the use of a limited amount of fluid but this is undesirable because small quantities of fluid produce different effects on different portions of the cornea. This effect is shown in Table E. The writer therefore used different fluids to flood the whole cornea and based his comparisons on the concentration of the drug and not on the quantity of the drug used.

In order to keep the duration of exposure constant the eye was thoroughly washed with saline at the end of the application of the drug. The duration of the subsequent anaesthesia was measured. The following sources of error deserve mention:-

(i) Tests on the anterior superior segments of the cornea are not reliable because the superior segment often is covered by folds of the upper conjunctiva and the anterior segment is covered by the nictitating membrane, hence the anaesthetic drug often fails to reach these portions. (See Table E).

Table E/

Table E.

1% Cocaine instilled for 1 min. (standard method) in the same rabbit (No. 4). The total capacity of the eye-pockets was 6-10 drops.

Duration of Anaesthesia in min. on the right eye.

Amount of fluid in drops	Inferio anterior quadrant	Inferio posterior quadrant	Superio anterior quadrant	Superio posterior quadrant
Cornea flooded 8-10	10-12	10-11	10-12	10-13
5	5-6	5-6	3-5	5-6
1	2-3	3-5	-	-

Results show that flooded cornea is more likely to give definite results than a constant amount of fluid and that inferio-posterior quadrant is more reliable for the test than other quadrants of the cornea.

(ii) /

(ii) There is considerable individual variation in the case with which anaesthesia of the cornea is produced in rabbits as already described. This error can be eliminated by making a series of tests on single rabbits but then it is important to allow an adequate interval between the tests. Usually a 24 hours' interval was allowed and in the case of the more important drugs an interval of from 3-5 days was allowed between tests. The decrease of sensitivity to threshold concentration of drugs to successive application is given in Table F.

Table F.

Effect of successive application on the left eye of rabbit (No. 3) of drug cocaine for 3 weeks (daily).

No.of days	Threshold conc. %	Time of duration in min.
1st	0.05	5 (incomplete and doubtful)
7th	0.10	5 (definite)
14th	0.25	15-20
21st	0.25	15-20

Results show that threshold concentration first increases, then remains constant if repeatedly used.

(b) Intradermal Wheal Method.

In this method the standard method of Rider was used as already described with a few necessary modifications. They are: (1) The amount of solution injected was 1/3 c.c. for the present method and 1/4 c.c. for standard experiments only, with a 1 c.c. syringe graduated to 1/100 c.c.; (2) The stimulus for pinprick being more definite was taken as a surer index for quantitative determinations; (3) The relative comparisons of the drug were made on their minimum effective concentrations though for the more important drugs a different method described in Parts I and III was used, and the effect of combining with adrenaline was also tested; (4) The injection of saline without any anaesthetic produces a short local anaesthesia, hence frequent controls with pure saline must be interposed amongst the experiments with drugs; (5) When solutions are used which are the minimum effective concentrations great care must be taken to wash the syringe thoroughly with the solution to be tested; (6) The factor of irritation was also measured by noting the pre-anaesthetic hypersthesia or post-anaesthetic/

anaesthetic pain or redness, at threshold concentrations or in relation to equal concentration and a rough estimation was made accordingly. Some of the drugs in this series did not show any redness or pain but showed a post-anaesthetic oedema and this was also included as irritation.

An example of results as obtained from the wheal method and its variability in ordinary dilutions is given as follows:-

Human Wheal.

Drug XVI/4. Conc. = 0.1%.

Reading No.	Duration of anaesthesia in min.
1	30-35
2	25-30
3	30-35
4	25-30
5	25-30
6	30-35
	27-32 Average
	$\sigma = 2.5$
	$\sigma_m = 1$

(c) /

(c) Anaesthesia of Frog's Sciatic Nerves.

The variables which must be controlled when this method is used are: - (1) Accidental damage of nerves during preparation; (2) Threshold stimulus for each preparation; (3) The possibility of fatigue of muscle-nerve preparation by undue frequency of stimulus, for which the conductivity of the nerve has been lowered by the drug; (4) Individual variations in size and seasonal variations.

Sollmann's technique as modified by Rider and already described, consists in leaving the nerves in situ (to avoid damage) and applying 1% cocaine to one side and a solution of the drug to be tested to the other side. The concentration of the drug tested is varied until one is found which produces paralysis in the same time as does the standard cocaine (although in some of his published results he gives equal concentration X time method). The strength of stimulus which was slightly above the threshold value is kept constant.

The author used a slight modification of this method. Rider made his solutions in distilled water, whereas the author used physiological saline, which is preferable for many reasons. The writer found/

found that with reasonable care it was easy to separate the sciatic gastrocnemius preparation without damage and hence used this. The presence of sheath over the sciatic nerve was found to interfere with the entrance of the drug and hence the nerve was carefully stripped. The preparation was isolated in the manner shown in Fig. 1 as described before in Part I.

The important point about this method is that the drug comes in contact only with the nerve and not with the muscle. Hence the method measures the paralysis of the nerve trunk and the nerve endings are not affected. Individual variations in frogs were minimised by using a standard solution of 1% cocaine on one nerve and the drug to be tested on the other nerve. The nerves were stimulated on platinum electrodes and care was needed to keep the electrodes moist and to keep a constant contact between the electrodes and the nerve and to avoid powerful stimuli which caused polarisation of nerves.

Preliminary experiments were made with cocaine to determine the method of electrical stimulation most favourable for obtaining of comparative results.

A/

A standard direct reading induction coil with two volt cell in the primary coil was used. The muscle movements were recorded with sensitive isometric levers mounted on stretched watch-spring which wrote on a smoked drum. The threshold stimulus usually lay between 20-50 volts, corresponding to 33-27.5 cm. of the secondary coil from the primary.

Threshold variability and the technique of its control.

Owing to the variability of the threshold stimulus of different frogs and also the two preparations of the same frog due to the difference in resistance in the two different secondary circuits (Fig. 1a) as well as the variable nature of the contact of the nerve to the electrodes, it was not possible to keep the secondary coil always at the same position for each threshold stimulus, but as it was thought necessary to do so it was done in the following manner, instead of interposing a high resistance in the secondary circuit as described by Laubender and Ost (1932).

The secondary coil was always kept at zero position of the scale, i.e. 33 cm. distance from the/

the primary and at right angles to it. At this position intensity of stimulus would be = 0. The secondary coil is now rotated to increase the intensity of stimulus to threshold intensity at a certain angle. This angle was noted and whenever the particular preparation was stimulated, it was so done by shifting the secondary coil up and down the scale at that particular angle, i.e. every preparation was stimulated at their respective angle and this technique helped to have a common start on the scale for all preparations.

(f) Tests of Toxicity.

The toxicity of the drug investigated was measured by intravenous injections into mice. Only those drugs which showed a reasonably potent local anaesthetic action were tested for toxicity. Preliminary toxicity tests were made by injecting about half a dozen mice in order to determine roughly whether or not the drug was violently toxic. An approximate value for the therapeutic ratio (activity/toxicity) was thus obtained. In the case of the more important drugs the median lethal dose was determined/

determined by injecting 50-100 mice with each drug according to Trevan's technique (1927) as follows:-

Mice weighing about 20 gm. (fasting weight) are kept at room temperature. They are injected with a definite dilute concentration of the drug 0.1%. Doses are varied by increasing or decreasing the volume of the fluid; injections are given at a uniformly slow rate. Data are taken at six months' interval to include seasonal variations, and the toxic dose calculated as mg. per kg. which kills 50% of the mice population. The tests of the present series have been carried out with only two modifications: (1) as the tests were carried within four months, the technique does not include seasonal variations to that extent, (2) survivals of populations were used again for a higher dose after a respite of one or two weeks.

Bibliography.

(1) Laubender and Ost. 1932. Arch. exp. Path. Pharmak. 165, 520.

(2) Rider, T.H. 1930. J. Pharmacol., Baltimore. 39, 329.

(3) Trevan, J.W. 1927. Proc. Roy. Soc. B.101, 483.